Research Article

The Step Further to Understand the Role of Cytosolic Phospholipase A₂ Alpha and Group X Secretory Phospholipase A₂ in Allergic Inflammation: Pilot Study

Ewa Pniewska,¹ Milena Sokolowska,² Izabela Kupryś-Lipińska,³
Monika Przybek,¹ Piotr Kuna,³ and Rafal Pawliczak¹

¹ Department of Immunopathology, Faculty of Biomedical Sciences and Postgraduate Training,
Medical University of Lodz, 7/9, Zeligowskiego Street, 90-752 Lodz, Poland

² Critical Care Medicine Department, Critical Center, National Institutes of Health, Bethesda, MD 20892-1662, USA

³ Department of Internal Diseases, Asthma and Allergy, Medical University of Lodz, 90-153 Lodz, Poland

Correspondence should be addressed to Rafal Pawliczak; rafal.pawliczak@umed.lodz.pl

Received 27 June 2014; Revised 11 August 2014; Accepted 11 August 2014; Published 27 August 2014

Academic Editor: Hiroto Matsuse

Copyright © 2014 Ewa Pniewska et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Allergens, viral, and bacterial infections are responsible for asthma exacerbations that occur with progression of airway inflammation. cPLA₂α and sPLA₂X are responsible for delivery of arachidonic acid for production of eicosanoids—one of the key mediators of airway inflammation. However, cPLA₂α and sPLA₂X role in allergic inflammation has not been fully elucidated. The aim of this study was to analyze the influence of rDer p1 and rFel d1 and lipopolysaccharide (LPS) on cPLA₂α expression and sPLA₂X secretion in PBMC of asthmatics and in A549 cell line. PBMC isolated from 14 subjects, as well as A549 cells, were stimulated with rDer p1, rFel d1, and LPS. Immunoblotting technique was used to study the changes in cPLA₂α protein expression and ELISA was used to analyze the release of sPLA₂X. PBMC of asthmatics released more sPLA₂X than those from healthy controls in the steady state. rDer p1 induced more sPLA₂X secretion than cPLA₂α protein expression. rFel d1 caused decrease in cPLA₂α relative expression in PBMC of asthmatics and in A549 cells. Summarizing, Der p1 and Fel d1 involve phospholipase A₂ enzymes in their action. sPLA₂X seems to be one of important PLA₂ isoform in allergic inflammation, especially caused by house dust mite allergens.

1. Introduction

Despite intensive studies on asthma pathogenesis and seeking the effective treatment, the number of new cases is increasing globally. Asthma is a very heterogeneous disease whose etiology has not been fully understood. Allergens, drugs, viral and bacterial infections, and stress are the most common factors that initiate and exacerbate asthma. About 70% of asthmatics are atopic [1]. The proposed mechanism of allergic asthma development suggests that allergen exposure causes sensitization, and continued exposure leads to airway hyper-responsiveness and inflammation. Airway inflammation primarily initiated as a defense process aiming to eliminate the damaging factor, which evolves to chronic state causing airway remodeling and impaired lung functions. In general

population of Lodz province (Poland) the most common sensitizing indoor allergens are house dust mites and cat [2]. Sensitivity to house dust mite and cat dander are risk factors associated with the development of asthma [3]. Many studies indicate that allergen exposure causes the exacerbation of asthma that occurs with impaired lung function and increases the need for hospitalization [4, 5].

Group X secretory phospholipase A₂ (sPLA₂X) has recently been investigated as one of the most important members of secretory PLA₂ in the inflammatory process [6]. Except its enzymatic activity sPLA₂ can act through the membrane receptors causing cell degranulation and initiating chemokines and cytokines production [7, 8]. Moreover, sPLA₂ can influence cytosolic PLA₂ (cPLA₂) action [9, 10]. In human airways a lot of resident cells (mast

cells, macrophages, endothelial cells, epithelial cells, and bronchial smooth muscle cells (SMC)) and haematopoietic cells (basophils, eosinophils, neutrophils, lymphocytes, and monocytes) are potential source of secretory phospholipases [11]. In asthmatics expression of sPLA₂-X predominates in airway epithelium. Moreover, both sPLA₂-X and sPLA₂-IIA are the main phospholipases detected in BAL fluid [6, 12]. sPLA₂-X and sPLA₂-XII are elevated in induced sputum cells of patients with asthma [13]. The studies with knockout mice showed that deficiency of sPLA₂-X reduced allergen-induced features of airway inflammation [14].

Cytosolic phospholipase A₂ group IVA (cPLA₂α) is the most potent enzyme in phospholipase A₂ superfamily catalyzing liberation of arachidonic acid (AA) from membrane phospholipids [15]. Our previous studies revealed that cPLA₂α participates in asthma pathogenesis [16]. What is more, rDer p1 caused overexpression of *PLA2G4A* in PBMC of asthmatics (unpublished data). Whalen et al. showed that PBMC of asthmatic patients stimulated with allergens in the presence of cPLA₂ inhibitor exhibited decreased production of proinflammatory cytokines [17]. cPLA₂ actions are mainly regulated by Ca²⁺ concentration and serine residue phosphorylation [18, 19]. LPS can modulate activity of cPLA₂ by phosphorylation [20]. Der p1 can activate MAPKs in different types of cells [21, 22]. Despite the abovementioned facts that prove the Der p1-cPLA₂α interactions, other mechanisms of allergens impact on lipid mediators remains not fully understood. Thus, we investigated whether allergens or LPS can directly stimulate the expression and/or phosphorylation of cPLA₂ protein in PBMC of severe asthmatics with atopic origin.

2. Material

2.1. Patients. Patients ($n = 7$) with severe asthma, who were allergic to house dust mite (Der p1) and cat (Fel d1) allergens, and healthy controls ($n = 7$) were enrolled to the study. The project was approved by the local ethics committee and an informed consent was obtained from every subject prior to the study. Patients were recruited from the Department of Internal Diseases, Asthma and Allergy of Medical University of Lodz. Asthma was recognized at least 6 months prior to the study and met the criteria of GINA Guidelines [23]. The severity of the disease was assessed according to the American Thoracic Society Workshop on Refractory Asthma 2000 Report [24]. All patients were classified as severe asthmatics. Patients were asked to not administer antihistamine drugs, oral glucocorticoids and leukotriene receptors antagonists 24 hours, and inhaled glucocorticoids and long-acting beta agonists 12 hours before blood drawing. Healthy volunteers had no known history of asthma or seasonal allergies. Table 1 presents characteristics of subjects enrolled to the study.

2.2. PBMC. Peripheral blood mononuclear cells (PBMC) were isolated using Histopaque 1077 (Sigma-Aldrich, St. Louis, MO), the density gradient cell separation medium according to the producer's instructions. Cells were cultured in RPMI1640 (Sigma-Aldrich, St. Louis, MO) with 10%

TABLE 1: Characteristics of patients with bronchial asthma and healthy subjects.

	Asthmatic patients	Healthy volunteers
<i>N</i>	7	7
Age (years)	48 (39–72)	36 (24–41)
Women/men	5/2	5/2
Smokers/nonsmokers	1/7	0/7
Inhaled GCS (μg/day) ^a	1508 (960–1600)	0
Systemic GCS (mg/day) ^b	18,8 (7–20)	0
FEV1 (%)	74.6	N/A
Allergic to Der p1	6/7	No
Allergic to Fel d1	3/7	No

^aDoses were calculated as budesonide equivalents. ^bDoses were calculated as prednisone equivalents.

heat-inactivated FBS (Sigma-Aldrich, St. Louis, MO) and antibiotics. 10 ng/mL of polymyxin B was added to medium used in allergen stimulation and 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO) for LPS incubation. 2×10^6 /mL PBMC were stimulated *in vitro* with LoTox deglycosylated recombinant *Dermatophagoides pteronyssinus* allergen 1 (rDer p1), LoTox deglycosylated recombinant *Felis domesticus* allergen 1 (rFel d1) (Indoor Biotechnologies, Cardiff, UK) or LPS from *E. coli*, serotype R515 (Enzo Life Sciences, NY). In dose-response systems three concentrations of allergens: 1 μg/mL, 5 μg/mL, and 10 μg/mL and LPS: 50 ng/mL, 100 ng/mL, and 500 ng/mL were tested (at 24 h). In time-course system 5 μg/mL of each allergen and 100 ng/mL of LPS were used and cells were collected in various time points: 0.5 h, 1 h, 2 h, 6 h, and 24 h.

2.3. A549 Culture. A549 cells, a human adenocarcinoma cell line, were obtained from the European Collection of Cell Cultures, Heath Protection Agency (Salisbury, UK) and were grown in Ham's F-12 K medium (Sigma-Aldrich, St. Louis, MO) with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), 2 mM of L-glutamine (Sigma-Aldrich, St. Louis, MO), 100 unit/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO). All experiments were performed when cells were 80% to 90% confluent. Cells were stimulated *in vitro* with LoTox deglycosylated recombinant *Dermatophagoides pteronyssinus* allergen 1 (rDer p1), LoTox deglycosylated recombinant *Felis domesticus* allergen 1 (rFel d1) (Indoor Biotechnologies, Cardiff, UK), or LPS from *E. coli*, serotype R515 (Enzo Life Sciences, NY).

3. Methods

3.1. Immunoblotting. Total protein from PBMC of patients with asthma, healthy subjects, and A549 cells was extracted in RIPA protein extraction buffer (Sigma-Aldrich, St. Louis, MO), supplemented with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). The lysate was centrifuged at 14,000 RPM and 4°C for 20 min, and the pellet discarded. Protein

concentrations were determined by the BCA Protein Assay Kit (Pierce Thermo Scientific, Rockford, IL) according to the manufacturer's protocol and using bovine serum albumin as a standard. 20 μ g of total protein was mixed with NuPAGE LDS Sample Buffer (Life Technologies, Carlsbad, CA) and in a 1:10 ratio with NuPAGE Reducing Agent (10x), heated for 10 min at 70°C. Protein samples were subjected to electrophoresis in 4–12% SDS-NuPAGE Gels (Life Technologies, Carlsbad, CA) at 200 V and electrophoretically transferred to a nitrocellulose membrane at 30 V for one hour. The membrane was blocked in 5% nonfat milk in TBST (20 mM Tris-HCL, 500 mM NaCl, 0.05% Tween 20, and pH 7.5) for 1 hour at room temperature. Then, the membranes were incubated for 12 h at 4°C with the one of the following antibody: polyclonal rabbit anti-cPLA₂ and anti-phospho-cPLA₂ (Ser505) and anti- β -actin antibodies (Cell Signaling, Danvers, MA). At the end of the overnight incubation, the membrane was washed with TBST and incubated for one hour in TBST containing the goat anti-rabbit IgG secondary antibodies conjugated with alkaline phosphatase (Sigma-Aldrich, St. Louis, MO). After incubation with secondary antibodies, the membrane was washed three times (3 $\times$ 5 mins) in TBST buffer. The band was developed using BCIP/NBT alkaline phosphatase substrate (Merck Millipore, Darmstadt, Germany). Densitometric analysis of bands was performed with Image J 1.34s software (Wayne Rasband, National Institutes of Health, Bethesda, MD) and the results are presented as fold change of optical density (OD).

3.2. ELISA. The sPLA₂X protein in supernatants from PBMC and A549 cells was measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kit (Cloud-Clone Corp., Houston, TX) according to the manufacturer's protocol. The limit of detection for sPLA₂X protein was 7.813 pg/mL.

3.3. Statistical Analysis. The data were analyzed using Statistica (v. 10.0; StatSoft, Tulsa, OK). Comparisons between groups were performed by using Mann-Whitney *U* tests or ANOVA followed by the Tukey's post hoc test when appropriate. Values of *P* < 0.05 were considered statistically significant.

4. Results

4.1. PBMC of Asthmatics Overproduced sPLA₂X in the Steady State. Hallstrand et al. [13] reported that sputum cells from asthmatics contained more sPLA₂X mRNA (qPCR) and protein (immunostaining) in comparison to controls, so we hypothesized that similar observation in PBMC is very possible. Taking into account the fact that sPLA₂X is produced as a zymogen and its cellular amount may not be relevant to its biological function we concluded that secretion of sPLA₂X will be the best approach to discover its potential involvement in asthma pathogenesis. The levels of sPLA₂X released by PBMC were compared in asthmatics and controls. The steady state concentration of sPLA₂X was significantly higher in

FIGURE 1: Concentration of sPLA₂X released by PBMC of asthmatics and healthy controls. 2×10^6 PBMC were incubated in medium containing RPMI1640 with 10% heat-inactivated FBS and antibiotics (37°C, 5% CO₂). Supernatants were collected after 24 hours of incubation in 37°C. The secretion of sPLA₂X was measured in indirect ELISA. Data are presented as mean $\pm$ SEM; **P* < 0.05 as comparison between studied groups.

asthmatics (411.09 pg/mL $\pm$ 129.2) than in healthy subjects (91.96 pg/mL $\pm$ 16.37) (Figure 1).

4.2. rDer p1 Stimulation Results in Different sPLA₂X and cPLA₂ Production Patterns in Asthmatics and Controls. PBMC of asthmatics and healthy subjects were stimulated with rDer p1 for 24 hours in three different concentrations: 1, 5, and 10 μ g/mL. There were no differences in sPLA₂X secretion between asthmatics and controls in any dose of rDer p1 whereas dust mite allergen in concentration of 10 μ g/mL significantly induced release of sPLA₂X (1.54 $\pm$ 0.24) when compared with relative protein expression of cPLA₂ α (0.79 $\pm$ 0.17) in asthmatics (Figure 2(a)). On the contrary, in healthy subjects, we did not observe similar fluctuation (Figure 2(b)).

4.3. Regulation of Relative cPLA₂ Protein Expression by LPS, rFel d1, and rDer p1 in PBMC in Time-Dependent Manner. Relative expression of cPLA₂ α protein was compared between healthy and asthmatic patients after stimulation with rDer p1 (5 μ g/mL), rFel d1 (5 μ g/mL), and LPS (100 ng/mL). cPLA₂ α basal expression was significantly lower in asthmatics as compared to healthy subjects. While we did not observe differences in cPLA₂ α protein synthesis between patients and controls after stimulation, there was a statistically significant increase of cPLA₂ α protein expression in PBMC of severe asthmatics in all tested time points after stimulation with rDer p1 when compared to steady state level. Also rFel d1 induced expression of cPLA₂ α protein after 0.5 h and 6 h of stimulation in patients. 24-hour incubation with LPS results in induction of cPLA₂ in asthmatics when compared with level before stimulation. In healthy subjects cPLA₂ α protein expression did not change significantly over time (Figures 3 and 4—blot).

FIGURE 2: sPLA₂X secretion and relative expression of cPLA₂α in PBMC of asthmatics (a) and healthy subjects (b) in response to rDer p1. PBMC (2×10^6) were stimulated with indicated doses of rDer p1 for 24 hours. *Control* represents cells treated with the vehicle. The *bar graph* shows the densitometry results for cPLA₂ (immunoblotting results) and ELISA results for sPLA₂X secretion. Data are presented as the fold change compared with the vehicle-treated cells. Data represent the mean $\pm$ SE from at least six independent experiments. **P* < 0.05 shows comparison between relative protein expression of cPLA₂ and secretion of sPLA₂X.

FIGURE 3: Relative cPLA₂α protein expression in PBMC from healthy subjects and asthmatic patients *in vitro* stimulated with rDer p1, rFel d1, and LPS in time-dependent manner. PBMC (2×10^6) were stimulated with 100 ng/mL LPS or rDer p1 (5 μg/mL) or rFel d1 (5 μg/mL) at the indicated time. *Control* represents cells treated with the vehicle. The immunoblot is representative of experiments in PBMC from at least six donors, each showing similar results. The *line graph* shows the densitometry results. Data are presented as the fold change compared with the vehicle-treated cells. The point "0" indicates cPLA₂ content in PBMC freshly isolated from blood, without culturing (untreated PBMC). Data represent the mean $\pm$ SE from at least six independent experiments. **P* < 0.05 shows comparison with untreated cells; **P* < 0.05 shows comparison between asthmatics and healthy subjects. HY: healthy subjects. SA: severe asthmatics.

FIGURE 4: Relative cPLA₂α protein expression in PBMC from healthy subjects and asthmatic patients *in vitro* stimulated with rDer p1, rFel d1, and LPS in dose-dependent manner. PBMC (2×10^6) were stimulated with indicated doses of LPS or rDer p1 or rFel d1 for 24 hours. Control represents cells treated with the vehicle. The immunoblot is representative of experiments in PBMC from at least six donors, each showing similar results. The line graph shows the densitometry results obtained from cells stimulated with rFel d1. Data are presented as the fold change compared with the vehicle-treated cells (control). Data represent the mean $\pm$ SE from at least six independent experiments. * $P < 0.05$ shows comparison between studied groups. HY: healthy subjects. SA: severe asthmatics.

4.4. cPLA₂ Protein Synthesis Is Diminished by rFel d1 in PBMC of Asthmatics. PBMC from asthmatics and healthy subjects were stimulated with rDer p1, rFel d1, and LPS in three different concentrations for 24 hours. While being not significant (as compared to control), there was a trend of increased relative expression of cPLA₂ in healthy subjects and decreased protein content in asthmatics after stimulation with rFel d1. However PBMC from asthmatics produced significantly less cPLA₂ (0.65 ± 0.15) than those from healthy subjects (1.61 ± 0.28) after stimulation with rFel d1 in concentration of 10 μg/mL (Figure 4).

4.5. LPS Induced cPLA₂ Phosphorylation in PBMC of Healthy Subjects. Phosphorylation of cPLA₂α was analyzed in PBMC stimulated with rDer p1 (5 μg/mL), rFel d1 (5 μg/mL), and LPS (100 ng/mL). The cells from controls (1.38 ± 0.22) contained more phosphorylated form of cPLA₂α than patients' PBMC (0.87 ± 0.1) after 2 h of incubation with LPS. The rapid change in phosphorylation of cPLA₂α was observed after 6 h stimulation with LPS (Figure 5). The allergens did not change the phosphorylation of cPLA₂.

4.6. Regulation of cPLA₂ Protein Synthesis by LPS, rFel d1, and rDer p1 in A549 Cells. In A549 culture the rFel d1 in concentration 10 μg/mL significantly decreased synthesis of

cPLA₂ (0.61 ± 0.01) (Figure 6). Any other stimulators did not change cPLA₂ expression in short time of incubation.

4.7. Recombinant Der p1 Induces Morphological Changes in A549 Cells. rDer p1 dose- and time-dependently caused morphological changes in A549 cells. Low concentrations and short incubation did not induce visible changes whereas higher concentrations and longer incubations led to cells shrinking and desquamation (Figure 7).

5. Discussion

We observed that asthmatics' PBMC released more sPLA₂X than control cells in the steady state. This observation is supported by previous reports, showing that asthmatics have increased content of sPLA₂X in airway epithelium, BALF, and sputum. The novelty of our study relates to regulation of different PLA₂ isoforms in response to rDer p1 stimulation. Interestingly rDer p1 in highest dose (10 μg/mL) significantly upregulated release of sPLA₂X protein when compared to relative cPLA₂α protein expression in asthmatics whereas in healthy subjects we did not observe this tendency. This observation suggests that sPLA₂X may be one of the important isoforms of PLA₂ in allergic response. Despite evidence that both enzymes cooperate in liberation of AA, sPLA₂X is also able to release AA independently to cPLA₂α [25]

FIGURE 5: Phosphorylation of cPLA₂α protein in PBMC from healthy subjects and asthmatic patients *in vitro* stimulated with LPS. PBMC (2×10^6) were stimulated with 100 ng/mL of LPS at indicated timepoints. *Control* represents cells treated with the vehicle. The immunoblot is representative of experiments in PBMC from at least six donors, each showing similar results. The *line graph* shows the densitometry results obtained from PBMC stimulated with LPS. Data are presented as the fold change compared with the vehicle-treated cells. Data represent the mean $\pm$ SE from at least six independent experiments. * $P < 0.05$ shows comparison between studied groups.

FIGURE 6: Relative cPLA₂α protein expression in A549 cells stimulated with rFel d1. A549 were stimulated with rFel d1 (5 μg/mL) for 24 hours. *Control* represents cells treated with the vehicle. The immunoblot is representative of three independent experiments in A549 cells, each showing similar results. The *bar graph* shows the densitometry results. Data are presented as the fold change compared with the vehicle-treated cells. Data represent the mean $\pm$ SE from at least three independent experiments. * $P < 0.05$ shows comparison with untreated cells.

and therefore may alone promote allergen-induced inflammation. Misso et al. suggested that increased activity of sPLA₂ may be associated with atopic status [26]. Some data

related to the role of sPLA₂X in airway inflammation come also from animal studies. Knock-in of human sPLA₂X to msPLA₂X^{-/-} mice restored allergen-induced inflammatory cell recruitment into airways as well as hyperresponsiveness to methacholine [27].

PBMC stimulated with rDer p1, rFel d1, or LPS showed increased production of cPLA₂α in comparison to steady state level in asthmatics but not in healthy subjects. The most rapid changes were observed after rDer p1 action, whereas 24-hour incubation with LPS was needed to induce significant increase of cPLA₂α content. The mechanism of Der p1 action is still not fully determined. Der p1 acts by PAR-2 receptor as well as in PAR-2-independent manner through activation of NF-κB and ERK1/2 [22, 28]. Activation of NF-κB pathway is prerequisite for cPLA₂ expression in many cell types [29–31], so this pathway can partially be involved in the induction of cPLA₂α expression. In our study we did not observe the changes in production of cPLA₂α between asthmatics and controls after stimulation with allergens and LPS (time-response scheme).

Dose-response scheme of our experiment showed that rFel d1 in highest dose (10 μg/mL) significantly decreased expression of cPLA₂α in asthmatics when compared to healthy subjects. The similar significant decrease of cPLA₂ synthesis was observed in A549 cells after stimulation with rFel d1. Fel d1 has been shown to may have enzymatic activity [32]. The structural analysis of Fel d1 revealed homology of the allergen with α-subunit of mouse salivary androgen

FIGURE 7: Morphological changes of A549 cells caused by rDerp1.

binding protein [33], uteroglobin and with the related Clara cell phospholipid binding protein, CC10 [34]. Uteroglobin is anti-inflammatory protein and can inhibit PLA₂ activity [35]. The similarity between rFel d1 and uteroglobin may suggest that the allergen has cytokine-like properties; thus it may be capable of inflecting the immune response [34].

Although Der p1 and Fel d1 are able to increase activity and phosphorylation of cPLA₂ in eosinophils [36], in our study we did not observe the significant changes in cPLA₂α phosphorylation after allergen stimulation. Only stimulation with LPS resulted in elevated level of phosphorylated cPLA₂α form in healthy subjects. PBMC of healthy volunteers respond better to LPS treatment. The diverse effects of LPS action have been observed earlier [37, 38]. Moreover it has been proved that LPS-induced cPLA₂ activity is TLR-4-dependent [39]. Different experiment systems and doses of LPS used in experiments seem to condition the results of LPS stimulation. In U937 cell line and macrophages LPS significantly increased the expression of cPLA₂ protein after 8 hours but not after 24 hours of stimulation whereas in tracheal smooth muscle cells the effective time points were 16 and 24 hours [30, 40].

rDer p1 in higher doses induces the desquamation of A549 cells. This effect was observed earlier and is result of

enzymatic activity of the allergen [28]. Der p1 is a cysteine protease able to degrade the occludin protein and ZO-1 protein in tight junction between epithelial cells [28, 41].

6. Conclusions

Results of the study showed that Der p1 and Fel d1 involve phospholipase A₂ enzymes in their action. sPLA₂X seems to be the more important PLA₂ isoform in airway inflammation, especially caused by house dust mite allergens. Der p1 has protease activity and can actively degrade and penetrate the epithelium barrier in airways. Moreover stimulation of sPLA₂X production whose activity is connected with further cysteinyl leukotrienes synthesis may sustain inflammatory process. This phenomenon might be supported additionally by decreased synthesis of cPLA₂ and subsequent diminished PGE₂ synthesis, which in respiratory track may also play protective role. Fel d1 seems to act rather by decreasing the cPLA₂ expression than induction of sPLA₂X. Further studies focusing on expression of different PLA₂ isoforms in different timepoints after inflammatory stimulus exposition should be analyzed to better understand the molecular mechanism of allergic inflammation.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The paper was supported from science budget for years 2010–2013 as research project (N N402 516939) and Medical University of Lodz Grants 502-03/0-149-03/502-04-004, 502-03/0-149-03/502-04-021, and 503/0-149-03/503-01. This paper was partially funded by Polish National Science Centre Grant no. DEC-2012/05/N/NZ5/02630.

References

- [1] WHO, "Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach," WHO, 2007.
- [2] I. Kupryś-Liphińska, A. Elgalal, and P. Kuna, "Skin prick test with inhaled allergens in the general population of Lodz province," *Pneumonologia i Alergologia Polska*, vol. 77, no. 3, pp. 229–234, 2009.
- [3] M. R. Sears, G. P. Herbison, M. D. Holdaway, C. J. Hewitt, E. M. Flannery, and P. A. Silva, "The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma," *Clinical and Experimental Allergy*, vol. 19, no. 4, pp. 419–424, 1989.
- [4] R. M. Green, A. Custovic, G. Sanderson, J. Hunter, S. L. Johnston, and A. Woodcock, "Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study," *British Medical Journal*, vol. 324, no. 7340, pp. 763–766, 2002.
- [5] C. S. Murray, G. Poletti, T. Kebabdzic et al., "Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children," *Thorax*, vol. 61, no. 5, pp. 376–382, 2006.
- [6] T. S. Hallstrand, Y. Lai, Z. Ni et al., "Relationship between levels of secreted phospholipase A₂ groups IIA and X in the airways and asthma severity," *Clinical and Experimental Allergy*, vol. 41, no. 6, pp. 801–810, 2011.
- [7] F. Granata, V. Nardicchi, S. Loffredo et al., "Secreted phospholipases A₂: a proinflammatory connection between macrophages and mast cells in the human lung," *Immunobiology*, vol. 214, no. 9-10, pp. 811–821, 2009.
- [8] F. Granata, A. Frattini, S. Loffredo et al., "Signaling events involved in cytokine and chemokine production induced by secretory phospholipase A₂ in human lung macrophages," *European Journal of Immunology*, vol. 36, no. 7, pp. 1938–1950, 2006.
- [9] A. N. Fonteh, G. Atsumi, T. LaPorte, and F. H. Chilton, "Secretory phospholipase A₂ receptor-mediated activation of cytosolic phospholipase A₂ in murine bone marrow-derived mast cells," *The Journal of Immunology*, vol. 165, no. 5, pp. 2773–2782, 2000.
- [10] S. Offer, S. Yedgar, O. Schwob et al., "Negative feedback between secretory and cytosolic phospholipase A₂ and their opposing roles in ovalbumin-induced bronchoconstriction in rats," *American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 288, no. 3, pp. L523–L529, 2005.
- [11] M. Triggiani, F. Granata, G. Giannattasio, and G. Marone, "Secretory phospholipases A₂ in inflammatory and allergic diseases: not just enzymes," *Journal of Allergy and Clinical Immunology*, vol. 116, no. 5, pp. 1000–1006, 2005.
- [12] Y. Lai, R. C. Oslund, J. G. Bollinger et al., "Eosinophil cysteinyl leukotriene synthesis mediated by exogenous secreted phospholipase A₂ group X," *The Journal of Biological Chemistry*, vol. 285, no. 53, pp. 41491–41500, 2010.
- [13] T. S. Hallstrand, E. Y. Chi, A. G. Singer, M. H. Gelb, and W. R. Henderson Jr., "Secreted phospholipase A₂ group X overexpression in asthma and bronchial hyperresponsiveness," *The American Journal of Respiratory and Critical Care Medicine*, vol. 176, no. 11, pp. 1072–1078, 2007.
- [14] W. R. Henderson Jr., E. Y. Chi, J. G. Bollinger et al., "Importance of group X-secreted phospholipase A₂ in allergen-induced airway inflammation and remodeling in a mouse asthma model," *Journal of Experimental Medicine*, vol. 204, no. 4, pp. 865–877, 2007.
- [15] C. C. Leslie, "Properties and regulation of cytosolic phospholipase A₂," *The Journal of Biological Chemistry*, vol. 272, no. 27, pp. 16709–16712, 1997.
- [16] M. Sokolowska, J. Stefanska, K. Wodz-Naskiewicz, M. Cieslak, and R. Pawliczak, "Cytosolic phospholipase A₂ group IVA is overexpressed in patients with persistent asthma and regulated by the promoter microsatellites," *Journal of Allergy and Clinical Immunology*, vol. 125, no. 6, pp. 1393–1395, 2010.
- [17] K. A. Whalen, H. Legault, C. Hang et al., "In vitro allergen challenge of peripheral blood induces differential gene expression in mononuclear cells of asthmatic patients: inhibition of cytosolic phospholipase A₂α overcomes the asthma-associated response," *Clinical and Experimental Allergy*, vol. 38, no. 10, pp. 1590–1605, 2008.
- [18] L. Lin, M. Wartmann, A. Y. Lin, J. L. Knopf, A. Seth, and R. J. Davis, "CPLA₂ is phosphorylated and activated by MAP kinase," *Cell*, vol. 72, no. 2, pp. 269–278, 1993.
- [19] J. H. Evans, S. H. Gerber, D. Murray, and C. C. Leslie, "The calcium binding loops of the cytosolic phospholipase A₂ C₂ domain specify targeting to Golgi and ER in live cells," *Molecular Biology of the Cell*, vol. 15, no. 1, pp. 371–383, 2004.
- [20] W. Tian, G. T. Wijewickrama, H. K. Jung et al., "Mechanism of regulation of group IVA phospholipase A₂ activity by Ser727 phosphorylation," *The Journal of Biological Chemistry*, vol. 283, no. 7, pp. 3960–3971, 2008.
- [21] C. K. Wong, C. B. Wang, M. L. Y. Li, W. K. Ip, Y. P. Tian, and C. W. K. Lam, "Induction of adhesion molecules upon the interaction between eosinophils and bronchial epithelial cells: Involvement of p38 MAPK and NF-κB," *International Immunopharmacology*, vol. 6, no. 12, pp. 1859–1871, 2006.
- [22] E. Adam, K. K. Hansen, O. A. Fernandez et al., "Erratum: The house dust mite allergen der p 1, unlike der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism," *Journal of Biological Chemistry*, vol. 282, no. 7, p. 5100, 2007.
- [23] Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma. In; 2011.
- [24] S. E. Wenzel, J. V. Fahy, C. Irvin et al., "Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions," *American Journal of Respiratory and Critical Care Medicine*, vol. 162, no. 6, pp. 2341–2351, 2000.
- [25] A. Saiga, N. Uozumi, T. Ono et al., "Group X secretory phospholipase A₂ can induce arachidonic acid release and eicosanoid production without activation of cytosolic phospholipase A₂

- alpha," *Prostaglandins and Other Lipid Mediators*, vol. 75, no. 1–4, pp. 79–89, 2005.
- [26] N. L. A. Misso, N. Petrovic, C. Grove, A. Celenza, J. Brooks-Wildhaber, and P. J. Thompson, "Plasma phospholipase A₂ activity in patients with asthma: association with body mass index and cholesterol concentration," *Thorax*, vol. 63, no. 1, pp. 21–26, 2008.
- [27] W. R. Henderson Jr., R. C. Oslund, J. G. Bollinger et al., "Blockade of human group X secreted phospholipase A₂ (GX-sPLA 2)-induced airway inflammation and hyperresponsiveness in a mouse asthma model by a selective GX-sPLA 2 inhibitor," *The Journal of Biological Chemistry*, vol. 286, no. 32, pp. 28049–28055, 2011.
- [28] H. F. Kauffman, M. Tamm, J. A. B. Timmerman, and P. Borger, "House dust mite major allergens Der p 1 and Der p 5 activate human airway-derived epithelial cells by protease-dependent and protease-independent mechanisms," *Clinical and Molecular Allergy*, vol. 4, article 5, 2006.
- [29] H. Hsieh, C. Wu, T. Hwang, M. Yen, P. Parker, and C. Yang, "BK-induced cytosolic phospholipase A₂ expression via sequential PKC- δ , p42/p44 MARK, and NF- κ B activation in rat brain astrocytes," *Journal of Cellular Physiology*, vol. 206, no. 1, pp. 246–254, 2006.
- [30] S. F. Luo, W. N. Lin, C. M. Yang et al., "Induction of cytosolic phospholipase A₂ by lipopolysaccharide in canine tracheal smooth muscle cells: involvement of MAPKs and NF- κ B pathways," *Cellular Signalling*, vol. 18, no. 8, pp. 1201–1211, 2006.
- [31] S. Luo, C. Lin, H. Chen et al., "Involvement of MAPKs, NF- κ B and p300 co-activator in IL-1 β -induced cytosolic phospholipase A₂ expression in canine tracheal smooth muscle cells," *Toxicology and Applied Pharmacology*, vol. 232, no. 3, pp. 396–407, 2008.
- [32] P. C. Ring, H. Wan, C. Schou, A. K. Kristensen, P. Roepstorff, and C. Robinson, "The 18-kDa form of cat allergen *Felis domesticus* 1 (Fel d 1) is associated with gelatin- and fibronectin-degrading activity," *Clinical and Experimental Allergy*, vol. 30, no. 8, pp. 1085–1096, 2000.
- [33] R. C. Karn, "The mouse salivary androgen-binding protein (ABP) alpha subunit closely resembles chain 1 of the cat allergen Fel dI," *Biochemical Genetics*, vol. 32, no. 7–8, pp. 271–277, 1994.
- [34] L. Kaiser, H. Grönlund, T. Sandalova et al., "The crystal structure of the major cat allergen Fel d 1, a member of the secretoglobulin family," *Journal of Biological Chemistry*, vol. 278, no. 39, pp. 37730–37735, 2003.
- [35] B. Chowdhury, G. Mantile-Selvaggi, L. Miele, E. Cordella-Miele, Z. Zhang, and A. B. Mukherjee, "Lys 43 and Asp 46 in α -helix 3 of uteroglobin are essential for its phospholipase A₂-inhibitory activity," *Biochemical and Biophysical Research Communications*, vol. 295, no. 4, pp. 877–883, 2002.
- [36] M. C. Seeds, K. K. Peachman, D. L. Bowton, K. L. Sivertson, and F. H. Chilton, "Regulation of arachidonate remodeling enzymes impacts eosinophil survival during allergic asthma," *The American Journal of Respiratory Cell and Molecular Biology*, vol. 41, no. 3, pp. 358–366, 2009.
- [37] M. L. Hernandez, M. Herbst, J. C. Lay et al., "Atopic asthmatic patients have reduced airway inflammatory cell recruitment after inhaled endotoxin challenge compared with healthy volunteers," *Journal of Allergy and Clinical Immunology*, vol. 130, no. 4, pp. 869.e2–876.e2, 2012.
- [38] E. Chun, S. Lee, E. Shim et al., "Toll-like receptor expression on peripheral blood mononuclear cells in asthmatics; implications for asthma management," *Journal of Clinical Immunology*, vol. 30, no. 3, pp. 459–464, 2010.
- [39] H. Qi and J. H. Shelhamer, "Toll-like receptor 4 signaling regulates cytosolic phospholipase A₂ activation and lipid generation in lipopolysaccharide-stimulated macrophages," *Journal of Biological Chemistry*, vol. 280, no. 47, pp. 38969–38975, 2005.
- [40] Y. J. Jiang, B. Lu, P. C. Choy, and G. M. Hatch, "Regulation of cytosolic phospholipase A₂, cyclooxygenase-1 and -2 expression by PMA, TNF α , LPS and M-CSF in human monocytes and macrophages," *Molecular and Cellular Biochemistry*, vol. 246, no. 1–2, pp. 31–38, 2003.
- [41] H. Wan, H. L. Winton, C. Soeller et al., "Quantitative structural and biochemical analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1," *Clinical and Experimental Allergy*, vol. 30, no. 5, pp. 685–698, 2000.