

Original article

Group II subfamily secretory phospholipase A₂ enzymes: expression in chronic rhinosinusitis with and without nasal polyps

Background: Group II subfamily secretory phospholipases A₂ (sPLA₂s) are the enzymes that can play a major role in inflammation. However, the presence of group II subfamily sPLA₂s in human sinonasal mucosa and their roles in chronic rhinosinusitis (CRS) are not well known. The purpose of this study was to investigate the expression of group II subfamily sPLA₂s in human sinonasal mucosa from controls and CRS patients with and without nasal polyps (NPs) and the regulation of expression by proinflammatory cytokines.

Methods: Surgical samples were investigated by means of reverse transcriptase polymerase chain reaction (RT-PCR) for evaluation of group II subfamily sPLA₂s mRNA expression, and the presence and location of group II subfamily sPLA₂s-positive cells were analyzed by means of immunohistochemistry. Furthermore, nasal explant culture and quantitative RT-PCR techniques were used to investigate the effect of interleukin (IL)-1 β and tumor necrosis factor (TNF)- α on group II subfamily sPLA₂s mRNA production in sinonasal mucosa.

Results: Messenger RNA expression of sPLA₂-IIA, -IID, and -IIE was significantly upregulated in tissues from CRS patients compared with control tissues. Among CRS patients, patients without NPs showed significantly stronger expression in sinonasal mucosa than patients with NPs of sPLA₂-IIA mRNA, and weaker expression of sPLA₂-IIE mRNA. Immunohistochemistry revealed enhanced protein expression of type II sPLA₂s and specific type IIA sPLA₂ in epithelial cells and submucosal glands in samples from CRS patients. Stronger type IIA sPLA₂ protein expression was found in samples from CRS patients without NPs when compared with NPs. Nasal explant culture experiments demonstrated that mRNA expression of sPLA₂-IIA, -IID, and -IIE was dramatically induced by IL-1 β and TNF- α .

Conclusions: The expression of some members of group II subfamily of sPLA₂s is upregulated in CRS and it may result from IL-1 β and TNF- α overexpression. Different individual group II subfamily sPLA₂s may play different roles in the pathogenesis of CRS with and without NPs.

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Key words: chronic rhinosinusitis; nasal polyps; phospholipase A₂; proinflammatory cytokine.

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Accepted for publication 5 March 2007

Chronic rhinosinusitis (CRS) is an extremely common disease that each year affects more than 31 million people in the United States alone (1). The most severe forms of CRS exhibit nasal polyps (NPs), with a high rate of symptomatic recurrence despite optimal medical and surgical care (2). The pathogenesis of CRS is poorly understood; however, genetic susceptibility, infection, anatomic abnormalities, and local immunologic imbalance have been postulated to play roles in its pathogenesis (2, 3). Recently, we have studied gene expression profiles in NP samples by means of DNA microarray and found Claracell 10-kDa protein gene was the one most down-regulated (4). Clara cell 10-kDa protein is a multifunction protein with anti-inflammatory and immunomodulatory effects (5). Although its functions are far from clear, one of its defined actions is to inhibit the activity of phospholipases A₂ (PLA₂s) (5). PLA₂ is the first enzyme in the

synthesis of two types of inflammatory lipid mediators with potent effects in the respiratory tract, namely the eicosanoids and platelet-activating factor (6). A number of mammalian PLA₂s have been identified to date, including 10 secretory PLA₂s (secretory phospholipases A₂, sPLA₂s; group IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XIIA), four cytosolic PLA₂s (cPLA₂s; group IVA, IVB, IVC, and IVD), and two intracellular Ca²⁺-independent PLA₂s (iPLA₂s; group VIA and VIB). (7) The sPLA₂ family represents a group of structurally related, disulfide-rich, low-molecular mass enzymes with a His-Asp catalytic dyad and strict Ca²⁺ dependence. Among sPLA₂ enzymes, sPLA₂-IIA, -IIC, -IID, -IIE, -IIF, and -V are clustered on human chromosome 1p34-36, so they are often referred to as the group II subfamily of sPLA₂s (7). While, sPLA₂-IIC, which is present in rodent testes, is not expressed as a functional protein in humans (7).

Increasing attention has been given to the group II subfamily of sPLA₂s, as these enzymes appear to be associated with inflammatory reactions. Group II subfamily sPLA₂s have been shown to be involved in the maturation, recruitment, and activation of inflammatory cells, such as macrophages, eosinophils, and platelets (8). Bronchial instillation of sPLA₂ has been shown to induce bronchoconstriction and tissue damage (9). Proinflammatory cytokines such as interleukin (IL)-1 β and tumor necrosis factor (TNF)- α can increase the synthesis and release of group II subfamily sPLA₂s in various cells (i.e. astrocytes, human airway epithelial cell lines, and synovial cells 7, 10, 11). However, at present, the role of group II subfamily sPLA₂s in the human nasal mucosa under normal and pathologic conditions has received little attention. Previous studies have showed that the activity of sPLA₂ with characteristics similar to sPLA₂-IIA was increased in nasal lavage fluid from allergic patients after allergen provocation (12). Group II sPLA₂s have been found in nasal and paranasal fluid and mucosa (13). Unfortunately, these studies have not investigated the role of different individual sPLA₂s. The increased sPLA₂ activity in pathologic conditions may not be equally attributed to any specific sPLA₂ type, and individual sPLA₂s display distinct enzymatic activities (7, 11). Recently, Lindbom et al. have found a large number of PLA₂ types in nasal epithelial cells from control and allergic rhinitis patients using reverse transcriptase polymerase chain reaction (RT-PCR; 14, 15). However, the expression and function of each group II subfamily sPLA₂ in CRS have not been investigated. Given our previous gene array results and the potential role of group II subfamily sPLA₂s in inflammation, we herein examined the expression of group II subfamily sPLA₂s in sinonasal tissues from control and CRS patients by means of RT-PCR and immunohistochemistry. Furthermore, we used nasal explant culture and quantitative RT-PCR techniques to assess the modulation of group II subfamily sPLA₂s mRNA expression in nasal mucosa by IL-1 β and TNF- α .

Methods

Subjects

This study was approved by the ethical committee of Tongji Medical College of Huazhong University of Science and Technology, and conducted with written informed consent from patients, in keeping with the mandate of the Declaration of Helsinki. Because of limitations in quantity, completely separate sets of sinonasal tissues were used for RT-PCR analysis, immunohistochemistry, and nasal explant culture experiments. Twenty-six and 74 patients who underwent functional endoscopic sinus surgery or septal surgery were enrolled for RT-PCR and immunohistochemistry experiments, respectively. These patients were divided into three groups: controls, CRS patients without NPs, and CRS patients with NPs. Clinical data of the patients are summarized in Table 1. Patients undergoing septopl-

Table 1. Patients' clinical data

	RT-PCR			Immunohistochemistry			
	Control group	CRS without NPs	CRS with NPs	Control group	CRS without NPs	CRS with NPs	Nasal explant culture
Number of subjects	6	10	10	19	28	27	10
Sex (male/female)	4/2	5/5	8/2	11/8	17/11	16/11	4/6
Age (years)	16–36	18–42	16–62	17–55	12–61	15–62	28–60
Number of patients with asthma	0	1	4	0	7	9	0
Number of patients with positive skin prick test results	0	4	3	0	10	13	0
Number of patients with aspirin sensitivity	0	0	0	0	0	0	0

CRS, chronic rhinosinusitis; NPs, nasal polyps; RT-PCR, reverse transcriptase polymerase chain reaction.

asty or rhinoseptoplasty because of anatomic variations and not having any sinus disease were considered control subjects and inferior turbinate mucosal samples were taken during surgery. CRS without and with NPs was diagnosed according to the clinical criteria by Meltzer et al. on the basis of history, clinical examination, nasal endoscopy, and sinus computed tomography (CT) scanning (3). Diseased sinus mucosal tissues and NP tissues were collected during surgery. Normal uncinate process mucosal tissues were obtained from 10 patients (eight patients with mucus retention cyst of maxillary sinus, one patient with mucocele of posterior ethmoid sinus, and one patient with mucocele of sphenoid sinus) undergoing sinus cyst resection and used for nasal explant culture. These patients did not show obvious anterior ethmoid inflammation on coronal CT scanning and endoscopy. All the subjects were skin-prick test-negative and none had a history of nasal blockage, nasal mucopurulent drainage, allergic disease, or asthma (Table 1). Light microscopy of all these specimens confirmed that they consisted of normal sinus mucosa without evidence of edema or inflammation.

In this study, subjects were excluded if they had received any oral steroid or antihistamine 3 months before the surgery. Topical medications were withheld for a minimum of 1 month before study. None had received antileukotrienes and immunotherapy. Medical management strategy was identical in both CRS groups. Human liver tissues affected by hepatitis C virus infection and gastric carcinoma tissues served as positive controls for RT-PCR and immunohistochemistry experiments.

RT-PCR

Total RNA was extracted from tissue samples by using TRI reagent (Molecular Research Center, Cincinnati, OH, USA) and treated by using a DNA-free kit (Ambion, Austin, TX, USA) to remove contaminating DNA. RNA concentrations were determined spectrophotometrically. Total RNA (0.5 μ g) was reverse transcribed to cDNA using random hexamer primers.

Amplification of each cDNA was performed with *Taq* DNA polymerase using gene-specific primers for the group II subfamily sPLA₂s and GAPDH as an internal control (Table 2). All primers used were constructed from published sequences (10, 14) and then synthesized by Bioasia Biotechnology Company (Shanghai, China). The PCRs were carried out as described (10, 14). The reaction cycle number was 35 for sPLA₂-IIA, -IID, and -IIE, 40 for sPLA₂-IIF and -V, and 30 for GAPDH. The PCR products were fractionated by agarose electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light. The density of each band was measured using ChemiImager™ 5500 Imaging System (Alpha Innotech Corp., San Leandro, CA, USA) to calculate the ratio of group II subfamily sPLA₂s mRNA to GAPDH mRNA. As negative controls, aliquots of each RNA were subjected to PCR without RT, and an aliquot of diethyl pyrocarbonate treated water was subjected to RT-PCR.

Tissue preparation, routine staining, immunohistochemistry, and quantification

Samples were fixed in formalin and embedded in paraffin. Paraffin sections (4 µm) were prepared from each block and stained with Giemsa and periodic acid-Schiff. Protein expression of group II subfamily sPLA₂s was examined by means of immunohistochemical staining. After deparaffinization and rehydration, sections were incubated with Target Retrieval Solution (Dako, Carpinteria, CA, USA). Afterwards, the sections were stained with anti-type II sPLA₂s (50 µg/ml) from Upstate (Lake Placid, NY, USA), anti-sPLA₂-IIA (30 µg/ml), and anti-sPLA₂-V (30 µg/ml) from Cayman Chemical (Ann Arbor, MI, USA). The anti-type II sPLA₂s antibody can detect all type II sPLA₂s, including sPLA₂-IIA, -IID, -IIE, and -IIF. The reaction was visualized through the use of the streptavidin-peroxidase complex method as described (16). Color development was achieved with 3', 3'-diaminobenzidine, which rendered positive cells brown. Species- and subtype-matched antibodies were used as negative controls.

Counting was carried out with a microscope eyepiece containing a 10 × 10 square reticule. Sections stained with Giemsa were used to count the number of eosinophils, mononuclear cells, and total infiltrating cells in the lamina proper. The sections were observed at 400× magnification, and 10 randomly selected fields were counted in a blinded fashion. Results were expressed as cells per square millimeter of lamina proper. Sections stained with periodic acid-Schiff were used to evaluate the number of goblet cells. Goblet cells were

observed in epithelium with same methods, but the results were expressed as cells per millimeter of epithelium. Quantitative measurement of group II subfamily sPLA₂s protein expression was analyzed using the HPIAS-1000 automated image analysis system (Olympus, Tokyo, Japan; 16, 17). Ten microscopic fields were randomly selected from each slide under 400× magnification. Results were presented as gray scores. The gray score reflects the optical density of selected field for analyzing. It was expressed as mean density per square micrometer. In this program, the gray score 0 represents black (no transmission) and 255 means white (full transmission). So, gray scores negatively correlated with the intensity of immunoreactivity. The gray score of background was measured in nontissue area and subtracted from the gray score of each selected field.

Nasal explant culture

Normal uncinate process mucosal tissue was obtained during surgery and sectioned into multiple samples of approximately 6 mm³. One was processed for histologic evaluation and the others were used for tissue culture as described previously (18). Briefly, sections of tissue were placed on 0.4-µm well inserts (Millipore Corp., Billerica, MA, USA) in 2 ml of defined medium (16). The tissue was oriented with the epithelium being exposed to the air, forming an air-liquid interface to mimic the *in vivo* situation. Tissue was incubated in the presence or absence of various concentrations of IL-1β or TNF-α (1, 10, and 100 ng/ml; R&D Systems, Minneapolis, MN, USA). The tissue was cultured at 37°C with 5% CO₂ in humidified air for 24 h.

Quantitative RT-PCR

After culture, tissue samples were homogenized, and RNA was extracted by using an RNeasy Mini kit (Qiagen, Valencia, CA, USA) and treated by using a DNA-free kit (Ambion) to remove contaminating DNA. cDNA was reverse transcribed from 0.5 µg of total RNA with random hexamer primers. Quantitative real-time PCR was performed on the LightCycler system (Roche Diagnostics, Mannheim, Germany) by using the SYBR Premix Ex Taq kit [TaKaRa Biotechnology (Dalian), Dalian, China] with the appropriate primers (Table 2) and samples according to the manufacturer's protocol. In brief, 2 µl cDNA was added to 10 µl 2× SYBR Premix Ex Taq master mix, 7.2 µl RNase-free water, and 0.4 µl of each primer (10 µM), resulting in a total volume of 20 µl. The PCR conditions consisted of an initial denaturation at 95°C for 30 s, followed by amplification for 45 cycles of 5 s at 95°C, 10 s at 55–65°C (varying between primer sets), and 15 s at 72°C. After PCR, a melting curve was constructed by increasing the temperature from 65 to 95°C with a temperature transition rate of 0.1°C/s. Relative gene expression was calculated by using the comparative CT method, as previously described (19), with GAPDH as a reference.

Statistical analysis

The proportion of CRS *vs* control patients and CRS patients without NPs *vs* with NPs having mRNA expression of group II subfamily sPLA₂s was compared using chi-squared test. Other data are presented as mean ± SD. Paired sets of RT-PCR and immunohistochemistry data were compared with ANOVA. The Spearman test was used to determine correlations. Paired *t*-test was used in tissue culture data analysis. Differences were considered statistically significant at a *P*-value of <0.05.

Table 2. Primer sequences used for RT-PCR analysis

Primer	Sequence	Expected product size (bp)
sPLA ₂ -IIA	[s] 5'-ATCCCCCAAGGATGCAAC-3' [a] 5'-AAAACAGGTGGCAGCAGC-3'	195
sPLA ₂ -IID	[s] 5'-ATCCTGAACCTGAACAAGATG-3' [a] 5'-AGTCGCTTCTGGTAGGTGTC-3'	328
sPLA ₂ -IIE	[s] 5'-GATGATCGAGAAGATGACAG-3' [a] 5'-AGCTTGTTGGGATAATGG-3'	324
sPLA ₂ -IIF	[s] 5'-GCCATCCTGCTTCGTG-3' [a] 5'-GGCAGTAGACATTGAGGAAGC-3'	309
sPLA ₂ -V	[s] 5'-GGGCTGCAACATTCGCACAC-3' [a] 5'-CCTCTCTCAGGAACCGAGCAG-3'	278
GAPDH	[s] 5'-GAAGGTGAAGGTCTGGAGTC-3' [a] 5'-GGAAGATGGTGATGGGATT-3'	227

sPLA₂, secretory phospholipases A₂; RT-PCR, reverse transcriptase polymerase chain reaction.

Results

Group II subfamily sPLA₂s mRNA expression in CRS

To investigate the pattern of gene expression for the group II subfamily sPLA₂s in CRS, we conducted RT-PCR analysis. We were not able to detect the mRNA expression of sPLA₂-IIF and -V in all the tissue samples after 40 cycled PCRs (results not shown). As illustrated in Fig. 1, a specific band for sPLA₂-IIA was observed in 83%, 80%, and 60% of tissue specimens from controls, and CRS patients without and with NPs, respectively. No statistically significant difference was found between groups. sPLA₂-IID mRNA expression rates in samples from controls and CRS patients without NPs were 33% and 70%, respectively, whereas it was 90% in samples from patients with NPs (*P* < 0.05 *vs* controls). No sPLA₂-IIE mRNA expression was found in control tissues, while its expression was detected in 60% (*P* < 0.05 *vs* controls) and 80% (*P* < 0.01 *vs* controls) of sinonasal mucosal tissues from CRS patients without and with NPs, respectively.

In order to compare the intensity of the positive expression, each group II subfamily sPLA₂/GAPDH ratio was calculated in different groups, and the results are shown in Table 3. Although the difference in expres-

sion rate of sPLA₂-IIA mRNA was not statistically significant between groups, tissue from CRS patients with and without NPs revealed a significantly higher mean density ratio for sPLA₂-IIA than did control mucosa (*P* < 0.01 for both). Moreover, compared with polyp tissue, tissue from CRS patients without NPs showed a significantly higher mean density ratio for sPLA₂-IIA (*P* < 0.01). The mean density ratio for sPLA₂-IID was significantly lower in control mucosa than in tissue obtained from CRS patients with and without NPs (*P* < 0.01 for both), and no statistically significant difference was found between specimens from CRS patients with and without NPs. As to sPLA₂-IIE, the mean density ratio was significantly higher in tissue from CRS patients with NPs compared with tissue from CRS patients without NPs (*P* < 0.05).

Histologic and immunohistochemical studies

Immunohistochemical studies were performed to detect and compare the presence and location of group II subfamily sPLA₂s protein in CRS and controls. On the basis of the availability of commercial antibodies, only type II sPLA₂s, and specific type IIA and V sPLA₂ protein expression was examined. Tissue sections were

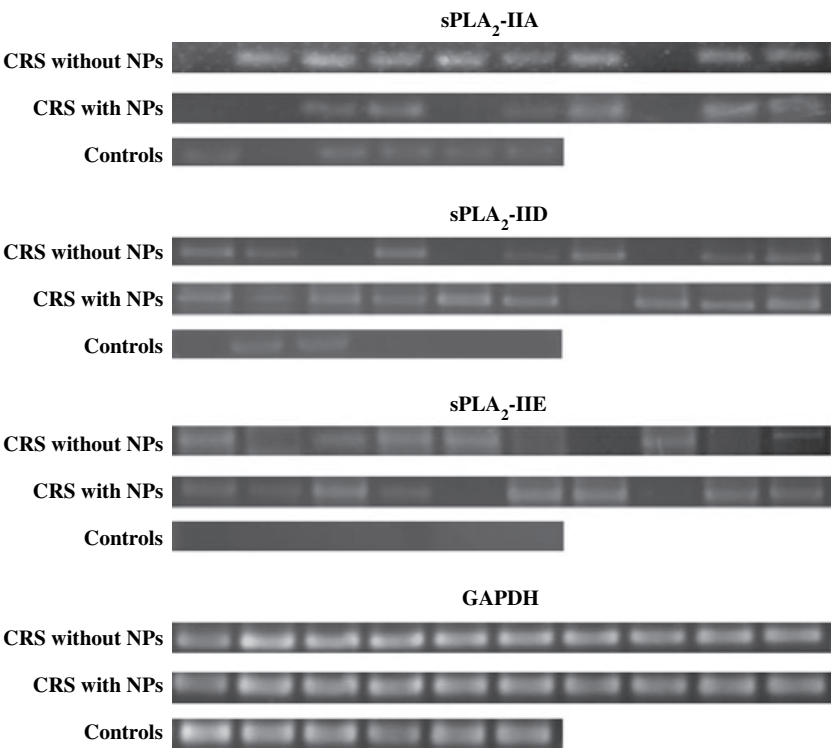


Figure 1. Reverse transcriptase polymerase chain reaction analysis of group II subfamily secretory phospholipases A₂ (sPLA₂s) mRNA in sinonasal mucosa of controls and chronic rhinosinusitis (CRS) patients with and without nasal polyps (NPs). sPLA₂-IIA, -IID, and -IIE mRNA expression was stronger in CRS sinus mucosa compared with that in control tissues. The polyp specimens revealed lower expression of sPLA₂-IIA mRNA, and higher expression of sPLA₂-IIE mRNA, than did specimens from CRS patients without NPs.

Table 3. Mean density ratio of positive band for each specific group II subfamily sPLA₂

Genes	Controls (N = 6)	CRS without NPs (N = 10)	CRS with NPs (N = 10)
sPLA ₂ -IIA	0.40 ± 0.01 (5)	0.79 ± 0.02 (8)*	0.57 ± 0.05 (6)*.##
sPLA ₂ -IID	0.41 ± 0.01 (2)	0.62 ± 0.05 (7)*	0.63 ± 0.08 (9)*
sPLA ₂ -IIE	0 (0)	0.50 ± 0.11 (6)*	0.66 ± 0.11 (8)*.##

In parentheses are numbers of positive bands on RT-PCR product electrophoresis. * $P < 0.01$ compared with controls; # $P < 0.05$ and ## $P < 0.01$ for CRS without NPs compared with NPs.

CRS, chronic rhinosinusitis; NPs, nasal polyps; sPLA₂, secretory phospholipases A₂; RT-PCR, reverse transcriptase polymerase chain reaction.

also stained with Giemsa and periodic acid-Schiff to assess the pattern and degree of inflammatory changes. Consistent with RT-PCR findings, staining for sPLA₂-V was negligible throughout the tissues, in spite of inflammation (results not shown). Type II sPLA₂s and sPLA₂-IIA protein expression could be found in the epithelium and submucosal glands of sinonasal tissues (Fig. 2). Control samples yielded no or weak staining for type II sPLA₂s and sPLA₂-IIA (Figs 2A,F and 3). In contrast, samples obtained from CRS patients with and without NPs showed significantly stronger staining for type II sPLA₂s and sPLA₂-IIA ($P < 0.05$ for all; Figs 2B–E,G–J and 3). When comparing polyp tissue and nonpolyp sinus tissue, no significant difference in expression intensity of type II sPLA₂s was found (Figs 2B–E and 3). However, significantly more positive staining of sPLA₂-IIA was observed in nonpolyp sinus tissue when compared with polyp tissue ($P < 0.05$; Figs 2G–J and 3). Analyzing the relationship between group II subfamily sPLA₂s staining intensity and the number of goblet cells and inflammatory

cells (the data for cell counts not shown), we found significant correlations between total type II sPLA₂s expression and goblet cells ($r = -0.89$, $P < 0.01$) and total infiltrating cells ($r = -0.75$, $P < 0.01$), but the correlations between total type II sPLA₂s expression and eosinophils ($r = -0.18$) and mononuclear cells ($r = -0.04$) did not reach statistical significance. Similarly, significant correlations were also observed between sPLA₂-IIA expression and goblet cells ($r = -0.64$, $P < 0.01$) and total infiltrating cells ($r = -0.50$, $P < 0.01$), no correlation was found between sPLA₂-IIA expression and eosinophils ($r = -0.01$) or mononuclear cells ($r = 0.04$).

Group II subfamily sPLA₂s gene expression in sinonasal tissue after IL-1 β and TNF- α stimulation

In order to determine the potential factors contributing to the regulation of group II subfamily sPLA₂s gene expression, normal uncinuate process mucosa was incubated in the absence or the presence of various concentration of IL-1 β or TNF- α . Individual group II subfamily sPLA₂s gene expression was examined after 24 h of incubation by means of quantitative RT-PCR. In sinonasal mucosa, sPLA₂-IIF and -V mRNA expression was barely detectable under all culture conditions, even after IL-1 β and TNF- α stimulation (results not shown). sPLA₂-IIA, -IID, and -IIE mRNA expression was very weak under control culture condition, but was markedly induced after stimulation with IL-1 β and TNF- α ($P < 0.05$ for all, compared with control condition; Fig. 4A,B). The effect of IL-1 β was more prominent than for TNF- α . The increase in sPLA₂-IID mRNA expression was higher than the increase in sPLA₂-IIA and -IIE mRNA expression.

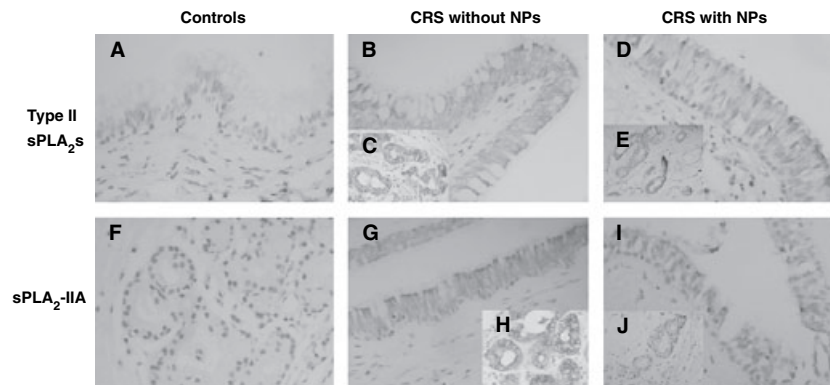


Figure 2. Representative photomicrographs of sinonasal tissue sections showing immunohistochemical staining. (A) No type II secretory phospholipases A₂ (sPLA₂s) staining in control nasal mucosa. (B and C) Type II sPLA₂s immunoreactivity in epithelial cells (B) and submucosal glands (C) of paranasal sinus mucosa from chronic rhinosinusitis (CRS) patients without nasal polyps (NPs). (D and E) Type II sPLA₂s immunoreactivity in epithelial cells (D) and submucosal glands (E) of NP tissues. (F) No sPLA₂-IIA staining in control nasal mucosa. (G and H) sPLA₂-IIA immunoreactivity in epithelial cells (G) and submucosal glands (H) of paranasal sinus mucosa from CRS patients without NPs. (I and J) sPLA₂-IIA immunoreactivity in epithelial cells (I) and submucosal glands (J) of NP tissues, the NP tissues immunostained less positively than did mucosa from CRS patients without NPs. Original magnification: $\times 200$.

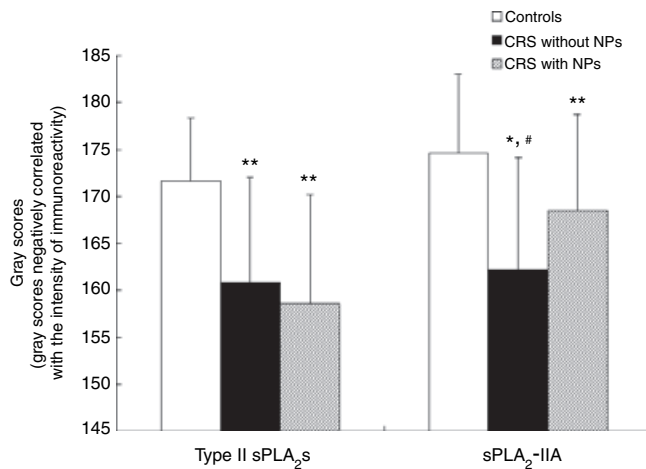


Figure 3. Quantification of protein expression of type II secretory phospholipases A₂ (sPLA₂s) and sPLA₂-IIA examined by means of immunohistochemistry in sinonasal mucosa from controls and chronic rhinosinusitis (CRS) patients with and without nasal polyps (NPs). Results were presented as gray scores. Gray scores negatively correlated with the intensity of immunoreactivity. * $P < 0.05$ and ** $P < 0.01$ compared with controls; # $P < 0.05$ for CRS without NPs compared with NPs.

Discussion

Group II subfamily sPLA₂s have been implicated in various biologic events, such as eicosanoids production, lipoprotein metabolism, and antibacterial defense (20), but their true physiologic functions and potential roles in the pathologic conditions still remain elusive. To date, very few published studies investigated the expression of individual group II subfamily sPLA₂s in tissues in human diseases (11, 21, 22). These studies have shown altered expression of group II subfamily sPLA₂s in lungs from patients with pneumonia, in livers affected by hepatitis C virus infection, and in gastric carcinoma tissues. In the present study, we have demonstrated weak mRNA expression of sPLA₂-IIA and -IID in some control nasal tissues and no expression of sPLA₂-IIE, -IIF, and -V in all six control nasal tissues. Some parts of our results were consistent with the report by Lindbom et al. (14). They did not find obvious mRNA expression of sPLA₂-IIF and -V in nasal epithelial cells from healthy subjects either. However, they detected mRNA expression of sPLA₂-IIA, -IID, and -IIE in all five subjects. This discrepancy could result from technical differences concerning the type of samples used and methods to harvest sample. In this study, we used surgical tissue samples which were immediately snap frozen in liquid nitrogen after taken; however, Lindbom et al. obtained nasal epithelial cells by a brush technique or nasal lavage which might alter the gene expression. Obviously, additional studies will

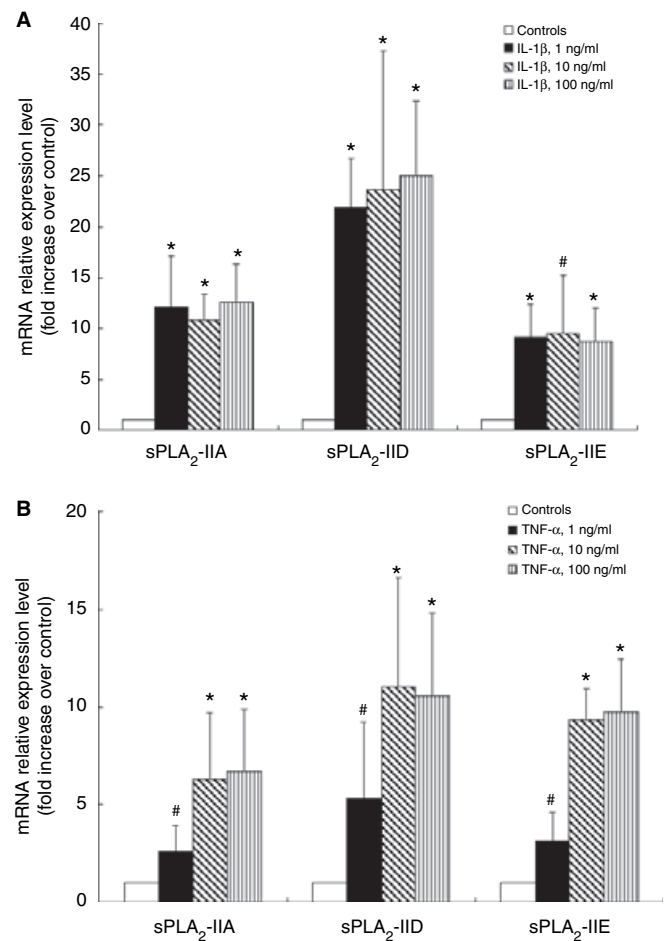


Figure 4. Treatment with interleukin (IL)-1β or tumor necrosis factor (TNF)-α induced a marked upregulation in expression of secretory phospholipases A₂-IIA, -IID, and -IIE mRNA in *ex vivo* cultured normal uncinat process mucosa. (A) Treatment with IL-1β ($n = 6-9$). (B) Treatment with TNF-α ($n = 6-7$). * $P < 0.01$ and # $P < 0.05$ compared with untreated mucosa.

be necessary to investigate this speculation. In paranasal sinus mucosa with CRS, we found that mRNA expression of PLA₂-IIA, -IID, and -IIE was significantly upregulated. Whereas even in the inflamed tissue obtained from CRS patients, we still could not detect the mRNA expression of sPLA₂-IIF and -V. In immunohistochemical studies, we found that type II sPLA₂s and sPLA₂-IIA protein was mainly expressed in the epithelial cells and submucosal glands of sinonasal mucosa. Consistent with our RT-PCR findings, control nasal tissue demonstrated scarce protein expression of type II sPLA₂s and sPLA₂-IIA, while paranasal sinus mucosa from CRS patients showed significantly increased expression; in addition, no protein expression of sPLA₂-V was detected in normal and diseased tissue. Hence, our RT-PCR and immunohistochemistry studies

demonstrated that specific members of group II subfamily sPLA₂s were upregulated in CRS and this expression profile was distinct from what was found in other diseases (11, 22), which indicates the expression profile of individual sPLA₂s may depend on the type of human tissue and pathology. Moreover, when comparing polyp tissue and nonpolyp sinus tissue, we found the polyp specimens revealed significantly lower expression of sPLA₂-IIA mRNA, and higher expression of sPLA₂-IIE mRNA, than did specimens from CRS patients without NPs. As to the protein expression, we found a significant increase in protein expression of sPLA₂-IIA, but not total type II sPLA₂s, in nonpolyp sinus tissue compared with polyp tissue, which strengthened our RT-PCR findings. These results indicate different individual group II subfamily sPLA₂s may play different roles in the pathogenesis of CRS with and without NPs. Although NPs and CRS are often taken together as one disease entity with NPs as a subgroup of CRS, increasing evidence suggests that CRS (without NPs) and NPs may be distinct disease entities (2, 3). For example, IL-5 was increased in NPs, but not in CRS (without NPs; 23); immunoglobulin E antibody formation to *Staphylococcus aureus* enterotoxins could be found in NPs, but not in CRS (without NPs; 24); and differences in transforming growth factor- β and matrix metalloproteinases might account for edema formation in NPs vs fibrosis in CRS (without NPs; 25, 26). Our present study showed CRS with and without NPs had distinct expression profiles of group II subfamily sPLA₂s, supporting the opinion that CRS with and without NPs may have different mechanisms underlying their pathogenesis and they might be two disease entities. So far the most important difference shown between NPs and CRS tissue without NPs is the eosinophilic infiltration in NPs. However, we did not find significant correlation between group II subfamily sPLA₂s expression and eosinophils. The molecular mechanism underlying different expression profiles between CRS with and without NPs needs to be further investigated. In addition, we found significant positive correlations between group II subfamily sPLA₂s expression and total inflammatory cells and goblets cells. It suggests that group II subfamily sPLA₂s may be involved in the inflammatory process in CRS; lipid mediator production, cytokine release promotion, and bacterial killing by group II subfamily sPLA₂s may coordinately promote inflammatory reactions and participate in the process that leads to the development of CRS. However, obviously, more studies are still needed to clarify the precise roles of individual group II subfamily sPLA₂s in the pathophysiology of CRS.

The factors contributing to the abnormal expression of group II subfamily sPLA₂s in sinonasal tissue are unknown. Previous studies have demonstrated that the expression of group II subfamily sPLA₂s in various cells

could be modulated by several cytokines such as IL-1 β , TNF- α , interferon- γ , and transforming growth factor- β (10, 11, 22, 27). Among these molecules, IL-1 β and TNF- α are two important proinflammatory factors, which are overexpressed in CRS and implicated in the pathogenesis of CRS (28). Therefore, it was possible to hypothesize that IL-1 β and TNF- α may be involved in the upregulation of group II subfamily sPLA₂s in CRS. As the group II subfamily sPLA₂s was not only expressed by nasal epithelial cells, but also by submucosal glands, we generated sinonasal mucosa explants and did *ex vivo* culture, which simulated the *in vivo* nasal environment. Consistent with conventional RT-PCR findings, expression of sPLA₂-IIA, -IID, and -IIE mRNA was weakly detected by means of quantitative RT-PCR under control culture condition, but was dramatically induced after treatment with IL-1 β and TNF- α , with 1 ng/ml IL-1 β and 10 ng/ml TNF- α causing maximal effects. The effect of IL-1 β was more prominent than for TNF- α . On stimulation with IL-1 β and TNF- α , sPLA₂-IIA, -IID, and -IIE mRNA demonstrated different fold increases, indicating different individual group II subfamily sPLA₂s genes may respond differently to these proinflammatory stimuli. In the present study, we could not find PLA₂-IIF and -V mRNA expression in sinonasal mucosal tissue under all culture conditions, even after treatment with very high concentration of IL-1 β or TNF- α (100 ng/ml). Our results suggest that the mRNA expression of sPLA₂-IIA, -IID, and -IIE, but not PLA₂-IIF or -V, can be induced by IL-1 β or TNF- α in sinonasal mucosa, and IL-1 β and TNF- α may contribute to the upregulated expression of sPLA₂-IIA, -IID, and -IIE in CRS. Besides IL-1 β and TNF- α , other cytokines, especially T_H2 cytokines (i.e. IL-4, IL-5, and IL-13), have also been implicated in the pathogenesis of CRS. Whether these cytokines are also responsible for the upregulation of group II subfamily sPLA₂s expression in sinonasal tissue requires further investigation. As the expression of T_H2 cytokines is more prominent in NPs than in CRS tissues without NPs, such investigation will also be helpful in understanding the factors contributing to the different expression profiles of group II subfamily sPLA₂s between CRS patients with and without NPs.

Conclusions

The expression of some members of group II subfamily sPLA₂s is upregulated in CRS and may be involved in the inflammatory process in CRS. Different individual group II subfamily sPLA₂s may play different roles in the pathogenesis of CRS with and without NPs. The upregulated expression of group II subfamily sPLA₂s may result from the elevated expression of IL-1 β and TNF- α in CRS. These results may provide potential targets for novel therapy of CRS.

Acknowledgments

The authors thank Dr Bruce Bochner for careful reading and editing of our manuscript. This study was supported by National

Nature Science Foundation of China (NSFC) grant 30500557 and scientific research foundation for the returned overseas Chinese scholars of State Education Ministry (SRF for ROCS, SEM) [2006]331 to Dr. Zheng Liu.

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