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Phospholipase A_2 mRNA expression in the nasal mucosa of healthy subjects and patients with seasonal allergic rhinitis*

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SUMMARY

Phospholipase A_2 (PLA_2) is a family of enzymes that play different role(s) in inflammation, but their importance in seasonal allergic rhinitis (SAR) has not been clarified. Here, we determined the levels of messenger ribonucleic acid (mRNA) for different PLA_2 types in the nasal mucosa of SAR patients (n=6) and healthy controls (n=5). Nasal brush samples were taken both during pollen season, when the symptoms of the patients were severe, and off-season, when the patients were free of symptoms. We found that PLA_2 IB, IIA, IID, IIE, IIF, III, IVA, IVB, IVC, VIA, VIB, VIIA, VIIB, VIIIA, VIIIB, X, XII and XIII were all expressed in each subject at both occasions. The mRNA levels of PLA_2 VIIA (platelet-activating factor (PAF) acetylhydrolase) were lower in SAR patients than controls, both during pollen season ($p = 0.03$) and off season ($p = 0.03$). These findings demonstrate that a large number of PLA_2 types are expressed in the nasal mucosa, regardless of whether there is ongoing allergic inflammation or not. The observation that PAF acetylhydrolase mRNA expression in the nasal mucosa is lower in SAR patients than in healthy subjects suggests the possibility that impaired ability to inactivate PAF might be of importance in SAR. Further studies are required to clarify whether the decreased PAF acetylhydrolase mRNA expression in SAR is accompanied by decreased enzyme activity and whether aberrations in PAF acetylhydrolase are present in infectious rhinitis patients as well.

Key words: seasonal allergic rhinitis, phospholipase A_2, platelet-activating factor acetylhydrolase, messenger RNA, nasal mucosa

INTRODUCTION

Phospholipase A_2 (PLA_2) is the first enzyme in the synthesis of two forms of lipid inflammatory mediators with potent effects in the respiratory tract: the eicosanoids and platelet-activating factor (PAF) (Barnes et al., 1998). Accordingly, PLA_2 hydrolyses the acyl ester bond at the *sn*-2 position of glycerophospholipids and releases arachidonic acid (AA), which is then metabolised to eicosanoids, and 1-*O*-alkyl-lysophosphatidylcholine (lyso-PAF), which is the precursor of PAF. At present, a number of different PLA_2s have been identified in human tissues: PLA_2 IB (Seilhamer et al., 1986), IIA (Seilhamer et al., 1989), IID (Ishizaki et al., 1999), IIE (Suzuki et al., 2000), IIF (Valentin et al., 2000a), III (Valentin et al., 2000b), IVA (Clark et al., 1991), IVB (Pickard et al., 1999), IVC (Underwood et al., 1998; Pickard et al., 1999), V (Chen et al., 1994), VIA (Larsson et al., 1998), VIB (Mancuso et al., 2000; Tanaka et al., 2000), VIIA (Tjoelker et al., 1995; Tew et al., 1996), VIIB (Hattori et al., 1996), VIIIA (Adachi et al., 1995), VIIIB (Adachi et al., 1997), X (Cupillard et al., 1997), XII (Gelb et al., 2000) and XIII (Takahashi). These are divided into two major groups: the cytosolic ($cPLA_2$) types (IVA, IVB, IVC, VIA, VIB, VIIB, VIIIA and VIIIB) which exert their functions inside the cell, and the secretory ($sPLA_2$) types (IB, IIA, IID, IIE, IIF III, V, VIIA, X, XII, and XIII) which can perform their biological activities extracellulary or on other cells. However, the precise contribution of these different PLA_2 types to the formation of inflammatory lipid mediators in the airways is not known. In addition, the role(s) of PLA_2s that are able to degrade PAF and so act rather as anti-inflammatory enzymes, have not been clarified (Tjoelker et al., 1995; Hattori et al., 1996; Tew et al., 1996). It is important, therefore, to study in more detail the diversity of PLA_2s in the airways and how the different PLA_2s

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may be activated during inflammation.

Previous investigations have demonstrated increased PLA_2 activity in nasal lavage fluid (NLF) from allergic patients after allergen provocation (Stadel et al., 1994; Touqui et al., 1994); both these investigations highlighted a secretory PLA_2 with characteristics similar to PLA_2 IIA. The IIA type of PLA_2 has also been determined in NLF from healthy subjects after methacholine provocation (Stadel et al., 1994). In addition to PLA_2 IIA, PAF acetylhydrolase-like activity has been detected in NLF, indicating the presence of PLA_2 VII (Touqui et al., 1994). We recently identified messenger ribonucleic acid (mRNA) transcripts for a large number of PLA_2 types (secretory IB, IIA, IID, IIE, III, V, VIIA and X, cytosolic IVA, IVB, IVC, VIA, VIB) in the nasal mucosa of healthy human subjects (Lindbom et al., 2001). The role of each of these PLA_2 types in the nasal mucosa is unknown. The mucosa is constantly exposed to environmental factors such as airborne particles, chemicals, allergens and microorganisms, and the presence of mRNA of several PLA_2 types may reflect a need for a fast response and a first line of defence against these various agents. Previous work has implicated PAF in allergic rhinitis (Leggieri et al., 1991; Albert et al., 1998). Accordingly, it can be hypothesised that increased expression of one or several of these PLA_2s is important to the release of AA and/or lyso-PAF in the nasal mucosa during inflammation, or that decreased expression of PLA_2(s) that are able to degrade PAF (PLA_2 VIIA, VIIB, VIIIA and VIIIB) may render the mucosa more liable to PAF-evoked inflammation and so play a role in rhinitis.

Here, we have examined the mRNA expression of different members of the PLA_2 family in the nasal mucosa of patients with seasonal allergic rhinitis (SAR) and healthy subjects. In addition, the mRNA levels of two postulated PLA_2 modulators, Clara Cell Secretory Protein (CCSP) and inducible nitric oxide synthase (iNOS) were investigated.

MATERIALS AND METHODS

Subjects

Eleven subjects volunteered for the investigation: six patients with seasonal allergic rhinitis (SAR) due to birch (2 male, 2 female) or grass pollen allergy (2 female) and five healthy subjects (2 male, 3 female) serving as controls. The inclusion criteria were: (*i*) a skin prick test verifying their pollen allergy but without signs of sensitisation to any perennial allergen in the SAR group and no signs of sensibilisation to any inhalant allergen in the control group; (*ii*) no history of a upper airway infection three weeks prior to examination and no signs of infection at anterior rhinoscopy; (*iii*) non-smoking habits. The skin prick test included inhalant allergen extracts: birch pollen, timothy pollen, pollen from *Artemisia vulgaris*, cat-, dog-, and horse dander, mites (*Dermphagoides pteronyssinus, Dermatophagoides farinae*) and moulds (*Alternaria, Cladosporium*) from ALK, Denmark. All eleven subjects were examined by anterior rhinoscopy, followed by a nasal brushing, at two different occasions; (*i*) during pollen season, and (*ii*) off pollen season. Thus, the examination confirmed ongoing SAR in the patient group during pollen season, but did not detect any signs of inflammation or irritation before nasal sampling at either occasion in the control group or at the off pollen season occasion in the SAR group. All subjects scored their rhinitis symptoms at each sampling occasion. Four different rhinitis symptoms (itching, sneezing, secretion, and stuffy nose) were scored from 0 (= no symptom) to 6 (= extremely troubled); these were then added together to give a combined nasal symptom score. All individuals gave their consent to participate after full information. The study was approved by the Ethics Committee at the University Hospital of Linköping, Sweden (Dnr 00-056).

Nasal mucosal samples

In order to verify ongoing allergic inflammation, cell materials from the nasal mucosa were obtained from one nasal cavity (by gentle brushing in the region between the inferior basal concha and the septum) and the materials examined for metachromatic cells and eosinophils. The materials were processed as previously described (Irander et al., 1997). Thus, after gently shaking the brush in saline, two glass slides were prepared by cytocentrifugation of two aliqouts of 0.2 ml each. One glass was prepared for analysis of metachromatic cells after fixation in methanol and staining with toluidin, and another glass for analysis of eosinophils, epithelial cells and neutrophils after fixation with ethanol and staining with Wright´s stain. To compensate for variations in the amount of cell materials between slides, eosinophil numbers were calculated as percent of the total numbers of identified cells from at least five different microscopic fields. Coding ensured a blinded analysis by light microscopy.

Detection of mRNA by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

After sampling, the nasal mucosal cells were rinsed from the brush in a phosphate saline buffer and centrifuged (250 g x 5 min). The cell pellets were immediately lysed and total cellular RNA isolated by the method of acid guanidium thiocyanate/phenol/chloroform extraction as previously described (Lindbom et al., 2001). RNA was reverse-transcribed into cDNA with Moloney Murine Leukemia Virus (MMLV) Reverse Transcriptase (SuperScript™ II, Life Technologies) according to the manufacturer's instructions. Total RNA was mixed with a buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$), 0.6 mM of each dNTP, and 0.5 mg oligo $(dT)_{18}$, and heated for 5 minutes at 65°C. After the incubation, the samples were chilled on ice and RT Super Script enzyme (200 units), RNasine (20 units) and DTT (100 mM) was added in a final volume of 20 µl. The samples were incubated for 60 minutes at 40°C followed by 5 minutes at 95°C. The cDNAs were stored at -20°C until used in PCR amplifications.

For PCR, 1 µl cDNA from each sample was used per amplifi-

cation. The reaction mixture contained 2 µl 10x PCR buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.6), 2 mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 mM oligonucleotide primers, and 0.5 units Taq DNA polymerase in a total volume of 20 µl. The primers have been described elsewhere (Lindbom et al., 2001),except for PLA_2 IIF [s] 5′-GCCATCCTGTCCTTCGTG-3′, [a] 5′-GGCAGTAGACATTGAGGAAGC-3′ (309 base pairs), VIIB [s] 5′-TGTGAGCCCACAGAGTGG-3′, [a] 5′-GCTCTACTGTCCAGGCTGC-3′ (257 base pairs), VIIIA [s] 5′-TAGCAGGTGGGATCGAGG-3′, [a] 5′-CGTCGGTATCCAGGAGCTG-3′ (261 base pairs), VIIIB [s] 5′-AGGTGAACGAGCTGGTACG-3′, [a] 5′-GTTGTGGGAAGGCAGCAG-3′ (191 base pairs), XII [s] 5′-ATTCCAGTATTGCCTCTCC-3′, [a] 5′-CTAGCTGTCGGCATCTCC-3′ (211 base pairs), XIII [s] 5′-GTATGGACTTGGGCATTCC-3′, [a] 5′-TGCACAGATGCAAGCTGC-3′ (265 base pairs), GAPDH [s] 5′-GGTCATCCATGACAACTTTGG-3′, [a] 5′-CGTCAAAGGTGGAGGAGTGG - 3′ (406 base pairs), iNOS [s] 5′-GGTGCACACAGCCTATTCC-3′, [a] 5′-CTGTCCTTCTTCGCCTCG-3′ (301 base pairs), and CCSP [s] 5′-CTCCACCATGAAACTCGCTG-3′, [a] 5′-GAAGAGAGCAAGGCTGGTGG-3′ (369 base pairs). The PCR reactions were carried out in a MJ-research (PTC-100) programmable thermal controller with an initial 2 min denaturation at 94°C followed by the cycled program 1 min at 94°C, 1 min at 65°C (62°C for CCSP) and 1 min at 72°C. The number of cycles used for amplification of the different PLA_2 types varied between 25 and 40 due to the relative abundance of the different PLA_2 types in the nasal mucosa (Lindbom et al., 2001). However, the same number of cycles was used to amplify a specific PLA_2 type in all samples, except PLA_2 VIIA and VIIB. Thus, the high relative abundance of PLA_2 VIIA and VIIB in samples taken off pollen season required only 30 and 25 cycles, respectively, to ensure exponential amplifications, as compared to 35 and 28 cycles during season. The number of cycles used for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) amplification was 20. A final extension of 7 min at 72°C ended the reaction. Ten µl from each PCR reaction was separated on a 1.5% agarose gel and ethidium bromide-stained. The stained gels were digitised into grey-scale images with a DC120 Zoom Digital Camera (Kodak Digital Science) and analysed with the Kodak Digital Science 1D Image Analysis Software. To compensate for variations in the number of cells isolated, efficiency of the mRNA isolation, and RT reaction between samples, the relative amount of amplified product in each sample were normalised the GAPDH gene.

Statistical determinations

Unless otherwise stated, values concerning the different groups are given as mean ± standard error of the mean (SEM). Mann-Whitney U test was used when comparing the mRNA results and cell count respectively, between groups, while Wilcoxon signed rank test was used when comparing symptom score.

RESULTS

Nasal symptom scores and cell findings

As expected, the nasal symptom score during pollen season was significantly higher in SAR patients than in controls (10.5 ± 2.0 vs 0 ± 0, $p = 0.004$), whereas there was no difference between the groups when examined off pollen season (1.0 ± 0.5 vs 0.2 ± 0.2, $p > 0.05$). Consistently, the score was significantly higher during pollen season than off-season in SAR patients (10.5 ± 2.0 vs 1.0 ± 0.5, $p = 0.03$) but not in controls (0 ± 0 vs 0.2 ± 0.2, $p > 0.05$).

Nasal mucosal samples stained by Wright´s stain admitted proper identification of eosinophils, epithelial cells and neutrophils. The median values of eosinophils during pollen season were 0.7% in SAR patients and 0.1% in controls. In samples taken off pollen season, the median values were reduced to 0% in both groups. In the SAR group, there was a correlation between the nasal symptom score and the number of eosinophils found during pollen season ($p = 0.08$, $r^2 = 0.7$).

Occasional metachromatic cells were found in the SAR patients during pollen season, correlating with the nasal symptom scores ($p = 0.05$, $r^2 = 0.8$). No such correlation was found during the off pollen period, neither for the eosinophils nor the metachromatic cells as the symptom scores was negligible in both groups.

Expression of different PLA_2s

Table 1 illustrates the mRNA expression of different PLA_2s in nasal mucosal cells from SAR patients and controls. In addition to the previously described PLA_2 mRNAs (Lindbom et al., 2001), the present investigation included mRNA from $sPLA_2$ IIF, XII and XIII, $cPLA_2$ VIIB, VIIIA and VIIIB. It thus appeared that all the $sPLA_2$ types, except for $sPLA_2$ V, and $cPLA_2$ types were expressed in the mucosal samples, regardless

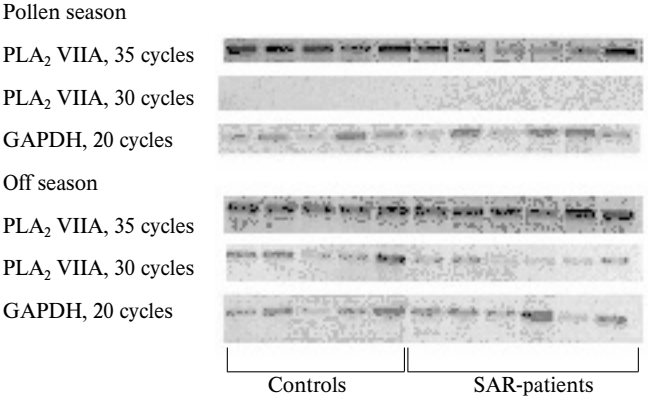

Figure 1. PLA_2 VIIA gene expression in nasal mucosal samples from SAR-patients and controls. The PCR-products were normalised to the "house-keeping" gene GAPDH to compensate for variations in the RNA isolation. When performing PCR on the samples taken off season, the plateau was reached using the same number of cycles as during the pollen season; therefore, a lower number of cycles had to be performed in order to stay within the exponential phase of the PCR-reaction.

Table 1. PCR analysis of the relative amounts of PLA_2 mRNAs present in nasal mucosal cell samples from SAR patients and controls. Each PCR sample was normalised to its GAPDH level. Values presented are mean ± SEM from six patients and five control subjects. ND = not detected.

	Pollen season			Off pollen season		
PLA_2 type	SAR	Control	p value	SAR	Control	p value
IB	1.2 ± 0.3	2.0 ± 0.4	0.26	2.6 ± 0.5	1.9 ± 0.5	0.43
IIA	1.6 ± 0.3	2.0 ± 0.6	0.53	1.5 ± 0.1	2.6 ± 0.5	0.13
IID	0.3 ± 0.2	0.6 ± 0.3	0.54	0.5 ± 0.2	0.3 ± 0.2	0.66
IIE	1.0 ± 0.3	2.0 ± 0.6	0.07	0.6 ± 0.1	1.0 ± 0.3	0.66
IIF	2.3 ± 1.3	3.3 ± 1.4	0.91	1.7 ± 0.3	3.4 ± 1.1	0.25
III	3.5 ± 1.1	4.9 ± 1.5	0.27	1.7 ± 0.3	2.9 ± 0.7	0.08
IVA	0.4 ± 0.1	0.4 ± 0.1	0.54	0.9 ± 0.2	0.8 ± 0.2	0.54
IVB	0.4 ± 0.1	0.2 ± 0.1	0.34	0.9 ± 0.1	0.5 ± 0.1	0.004
IVC	0.9 ± 0.1	0.6 ± 0.2	0.27	1.0 ± 0.3	1.5 ± 0.1	0.66
V	ND	ND	---	ND	ND	---
VIA	1.1 ± 0.3	2.1 ± 0.6	0.20	1.1 ± 0.4	1.3 ± 0.6	0.66
VIB	1.2 ± 0.2	0.9 ± 0.2	0.27	0.9 ± 0.2	0.8 ± 0.2	0.79
VIIA	0.9 ± 0.3	2.4 ± 1.1	0.03	8.5 ± 0.1	17.3 ± 0.2	0.03
VIIB	1.6 ± 0.2	2.1 ± 0.6	0.90	0.6 ± 0.1	1.1 ± 0.1	0.01
VIIIA	1.9 ± 0.9	1.4 ± 0.1	0.90	2.6 ± 0.7	2.5 ± 0.9	0.88
VIIIB	1.2 ± 0.5	1.4 ± 0,4	0.70	1.9 ± 1.1	1.5 ± 0.6	0.66
X	1.9 ± 0.3	2.6 ± 0.2	0.07	1.2 ± 0.2	1.3 ± 0.2	0.79
XII	2.7 ± 0.9	4.2 ± 1.3	0.26	1.5 ± 0.3	1.1 ± 0.2	0.66
XIII	0.1 ± 0.1	0.2 ± 0.1	0.56	0.6 ± 0.1	0.6 ± 0.2	0.79
iNOS	3.8 ± 1.9	1.2 ± 0.2	0.10	1.9 ± 0.2	2.2 ± 0.3	0.33
CCSP	1.2 ± 0.2	1.3 ± 0.2	0.79	1.1 ± 0.2	1.2 ± 0.1	0.79

of if they were from patients with or without ongoing SAR, or from controls (Table 1).

As shown in Figure 1 and Table 1, the mRNA levels of $sPLA_2$ VIIA (PAF acetylhydrolase) were lower in SAR patients than in controls. This was the case both during pollen season ($p = 0.03$) and off-season ($p = 0.03$). Furthermore, the mRNA levels of $cPLA_2$ VIIB (PAF acetylhydrolase II) were lower in SAR patients than in controls in samples taken off-season ($p = 0.01$), but not in samples taken during season ($p = 0.90$). By contrast, the mRNA levels of $cPLA_2$ IVB were higher in SAR patients than in controls in samples taken off-season ($p = 0.004$), but not in samples taken during season ($p = 0.34$). No significant differences in mRNA levels of other PLA_2 types were found between SAR patients and controls.

Expression of iNOS and CCSP

The level of iNOS mRNA was higher in SAR patients (3.8±1.9) than controls (1.2±0.2) during pollen season, but the p value ($p = 0.1$) did not reach statistical significance due to large variation between the individuals. No difference between the two groups was seen during off-pollen season (SAR patients 1.9±0.2, controls 2.2±0.3, $p = 0.33$).

The mRNA level of CCSP did not show any difference between SAR-patients (1.2±0.2) and controls (1.3±0.2), neither during pollen season ($p = 0.79$), nor off pollen season (SAR patients 1.1±0.2, controls 1.2±0.1, $p = 0.79$).

DISCUSSION

The object of this investigation was to compare the nasal PLA_2 mRNA pattern during allergic inflammation with that during normal non-inflammatory conditions. It was essential, therefore, to ascertain that the SAR patients had ongoing allergic inflammation during pollen season (but not off-season) and that the controls had no inflammation. Accordingly, the symptom score, the visual examination, and the increased numbers of eosinophils verified an allergic inflammation in the SAR patients during pollen season, while there were no symptoms or signs of inflammation off pollen season. Controls showed no signs of allergic or other inflammation at either occasion.

The mRNA levels of $sPLA_2$ VIIA (PAF acetylhydrolase) were lower in SAR patients than in controls both during pollen season and off-season (Table 1). This suggests that the nasal mucosa of SAR patients is less well equipped for degrading PAF than is the mucosa of healthy subjects. The reason for such a difference remains to be clarified, as does the finding that the mRNA levels of PAF acetylhydrolase were considerably lower during pollen season than off season in both groups investigated. Previous investigations have demonstrated an increased gene expression of interleukin 4 in the nasal mucosa of SAR patients during the pollen season (Karlsson et al., 1995) and a decrease of interferon-α mRNA in the blood during the summer in healthy subjects of the Finnish population (Katila et al., 1993). It is possible that the gene expression of $sPLA_2$ VIIA, like that of interferon-α, is season-dependent. As to the lower $sPLA_2$ VIIA mRNA expression in SAR patients, it can be anticipated that, during pollen season and mucosal inflammation, the gene expression of PAF acetylhydrolase may be down regulated by pro-inflammatory cytokines or other agents. Several studies have been performed to investigate the regulation of PAF acetylhydrolase gene expression and activity, both *in vitro* studies on cell lines and *in vivo* studies on animals (Tjoelker and Stafforini, 2000). According to experiments on human cells, PAF acetylhydrolase is downregulated by lipopolysaccharide and interferon-γ, and by a number of other cytokines, while PAF induces expression and secretion of PAF- acetylhydrolase (Cao et al., 1998; Kawano et al., 1999). However, the finding that the PAF acetylhydrolase expression was lower in SAR patients also during off pollen season, i.e. at a time when there were no signs or symptoms of mucosal inflammation, suggests that other factors than inflammatory mediators may be responsible. Further studies must be undertaken to examine whether there are genetic or other factors in SAR patients that may explain the low expression of PAF acetylhydrolase in the nasal mucosa. Mutations in the PAF acetylhydrolase gene have previously been associated with severe asthma in Japanese individuals (Miwa et al., 1988; Stafforini et al., 1996) and two genetic variants of PAF acetylhydrolase seem to play a key role in atopic and asthmatic processes in Caucasian populations (Kruse et al., 2000). Moreover, acetylhydrolase activity in bronchoalveolar lavage fluid (BALF) and serum is significantly decreased in patients

with bronchial asthma (Tsukioka et al., 1996; Triggiani et al., 1997) indicating that decreased PAF acetylhydrolase expression may play a general role in airways inflammation.

The mRNA levels of the $cPLA_2$ VIIB (PAF acetylhydrolase II) were lower in SAR patients than controls in samples taken off-season ($p = 0.01$), but not in samples taken during pollen season ($p = 0.90$). There is presently no explanation for the difference between the two groups off-season, and subsequent studies will have to examine whether there are genetic or other factors in SAR patients that may explain this finding as well. $cPLA_2$ VIIB has been proposed to play a protective role not only by hydrolysing PAF, but also by removing oxidised phospholipids in the cell membrane caused by oxidative stress (Matsuzawa et al., 1997). It is possible, therefore, that the relative increase in $cPLA_2$ VIIB mRNA levels in SAR patients during pollen season is evoked by lipid peroxidation processes in the nasal mucosa.

Aside from PLA_2 VII, we found that $sPLA_2$ IB, IIA, IID, IIE, IIF, III, X, XII and XIII, as well as $cPLA_2$ IVA, IVB, IVC, VIA, VIB, VIIIA, VIIIB, were all expressed in each subject at both sampling occasions. These findings demonstrate that a large number of PLA_2 types are expressed in the human nasal mucosa, both under normal and inflammatory conditions. The mRNA levels of PLA_2 IVB were higher in SAR patients than controls in samples taken off-season, but not in samples taken during season. The biological function of $cPLA_2$ IVB is unknown, but because this enzyme has preference to cleave phospholipids at the *sn*-1 position (Song et al., 1999) it is perhaps not primarily involved in AA release or PAF metabolism.

The finding that there were no significant differences in mRNA levels of other PLA_2 types between SAR patients and controls speaks against a major role for these enzymes in SAR. It should be emphasised, however, that RT-PCR may not be able to demonstrate small differences in mRNA expression, and so it is not justifiable to draw firm conclusions regarding the absence of differential expression. Moreover, a lack of difference in mRNA levels does not preclude differences in protein levels or enzyme activity, and further studies taking these considerations into account must be undertaken to clarify the role of different PLA_2s in SAR.

Thus, the different PLA_2 enzymes have a wide variety of biological functions that should be further explored in SAR patients. For example, the PLA_2 II types posses bactericidal activity and are inducible by bacterial lipopolysaccharide in mice and rats (Ishizaki et al., 1999; Koduri et al., 2002; Suzuki et al., 2000) and also the V, X and XII types have bactericidal properties (Koduri et al., 2002). $cPLA_2$ IVA is strongly associated with AA liberation and eicosanoid production in many experimental systems, including mice that are genetically deficient in the IVA enzyme (Bonventere et al., 1997; Uozomi et al., 1997). $cPLA_2$ VIA, which has previously been proposed as a house-keeping enzyme involved in phospholipid remodelling and homeostasis, was recently found to have signalling functions (Ma et al., 2001). Future investigations will have to address whether these or other functions of PLA_2 are affected in the nasal mucosa of SAR patients.

CCSP (also known as CC10 or CC16) is suggested to be a natural PLA_2 inhibitor (Broeckaert and Bernard, 2000) and decreased CCSP levels were recently demonstrated in NLF of children with allergic rhinitis (Lindahl et al., 2001). The present study, however, did not show any difference in CCSP mRNA levels between SAR patients and controls. Neither was there any statistical difference in the mRNA levels of iNOS, an enzyme that is upregulated by $sPLA_2$ II (Baek et al., 2001). This contrasts with a report claiming that the greater amounts of NO in the nasal air of rhinitis patients (Kharitonov et al., 1997) are due to increased nasal iNOS expression (Kawamoto et al., 1999). On the other hand, no expression of iNOS protein was recently found in eosinophils from patients with allergic rhinitis, while there was strong expression in healthy controls (Ferreira et al., 2002). In our study, the mean level of iNOS mRNA was higher in SAR patients than controls during pollen season, but there was great variation between individuals and the p value for difference between the groups ($p = 0.1$) did not reach statistical significance. This could be explained by the fact that RT-PCR is variable and that the power in our study was low. Notably, other investigators using RT-PCR have also found large variation in the gene expression of iNOS in the nasal mucosa of allergic subjects (Takeno et al., 2001). On the other hand, the possibility that another NO-producing enzyme, neuronal NOS (nNOS), might be responsible for the increased exhaled NO levels in allergic rhinitis should not be overlooked (Olthoff et al., 2002). A larger study of a homogeneous group of SAR patients is warranted, preferably using real-time PCR for quantifying the different NOS mRNA levels in the nasal mucosa.

In summary, this study shows that a large number of PLA_2 types (including the newly discovered $sPLA_2$ IIF, XII, and XIII) are expressed in the human nasal mucosa, regardless of whether there is ongoing allergic inflammation or not. The observation that PAF acetylhydrolase mRNA expression in the nasal mucosa is lower in SAR patients than in healthy subjects points to the possibility that impaired ability to inactivate PAF might be of importance in SAR. The suggestion that PAF is involved in SAR (Leggieri et al., 1991; Albert et al., 1998) is thus gaining some further support. If PAF is more active in SAR patients deficient in PAF acetylhydrolase, this should be taken into account in clinical studies of PAF antagonists.

The present work should be regarded as a pilot study. An investigation with a larger number of subjects is necessary to establish if these findings apply to the majority of SAR patients. Real-time PCR could also reveal differences in the gene expression between SAR-patients and non-allergic subjects that are not detectable using traditional PCR. Attention must also be paid to the possibility that impairment of PAF-AH plays a role not only in SAR but also in infectious rhinitis.

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