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Role of prostaglandin D₂ and E₂ terminal synthases in chronic rhinosinusitis

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Summary

Background Prostaglandin (PG)D₂ and E₂, two major cyclooxygenase (COX) products, are generated by PGD₂ synthase (PGDS) and PGE₂ synthase (PGES), respectively, and appear to mediate airway inflammation.

Objective We sought to determine the role of PGDS and PGES in the pathophysiology of chronic rhinosinusitis (CRS).

Methods The study examined the expression of PGDS and PGES in nasal polyps of 22 CRS patients. As controls, uncinate process mucosae were obtained from 12 CRS patients not having nasal polyps and five subjects without sinusitis. Immunohistochemistry and quantitative real-time PCR were used to evaluate the expression.

Results Both PGDS and PGES were detected in nasal polyps by immunohistochemistry. Significantly greater levels of PGDS mRNA and lesser levels of PGES mRNA were observed in the nasal polyps as compared with uncinate process mucosae, and an inverse correlation between PGDS and PGES expression was observed. Levels of PGDS mRNA in nasal polyps were positively correlated with degree of infiltration by EG2+ eosinophils, whereas the levels of PGES were inversely correlated. Significantly increased levels of PGDS and conversely decreased levels of PGES were observed in asthmatics as compared with non-asthmatics. In addition, PGDS and PGES levels were positively and inversely correlated with the radiological severity of sinusitis, respectively.

Conclusions These results suggest that PGDS and PGES display an opposite and important role in the pathophysiology of CRS such as polyp formation, and more specifically, a biased expression of these synthases might contribute to the development of CRS by affecting eosinophilic inflammation.

Keywords eosinophil, PGD₂, PGE₂, sinusitis, synthase

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Introduction

Prostaglandins (PGs) are lipid mediators that regulate immune function [1]. Among these, PGD₂ and PGE₂ are thought to mediate airway inflammation [2]. PGD₂ mediates a variety of biological activities, including vasodilation and bronchoconstriction [3]. Overproduction of PGD₂ induces eosinophilic lung inflammation, and deletion of DP, a PGD₂ receptor, reduces eosinophilic inflammation in murine models of asthma [4, 5]. PGE₂ has a pro-inflammatory role by enhancing eosinophil survival and mucin gene expression [6–8]. However, PGE₂ also has anti-inflammatory activity and halts the uncontrolled

synthesis and release of cysteinyl leukotrienes from mast cells and inhibits airway smooth muscle cell proliferation [9–12]. Thus, it remains unclear whether PGE₂ are deleterious or beneficial in the pathogenesis of airway inflammation.

PGD₂ and PGE₂ are derived from arachidonic acid (AA). AA is mobilized from membrane phospholipids by phospholipases and is converted to PGH₂ by cyclooxygenase (COX) enzymes. PGH₂ is acted upon by PGD₂ synthase (PGDS) and PGE₂ synthase (PGES) to produce PGD₂ and PGE₂, respectively [1]. Two distinct types of PGDS exist: lipocalin-type PGDS (l-PGDS) and haemopoietic-type

PGDS (h-PGDS) [13, 14]. A number of PGES enzymes also exist [15]. Among these, microsomal PGES-1 (m-PGES-1) preferentially combines with the inducible form of COX-2 and is believed to generate PGE₂ under a range of inflammatory conditions [15, 16]. Although information regarding the role of COX in the pathogenesis of respiratory allergy is accumulating [17–20], it is not yet clear whether these synthases regulate airway inflammation.

Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory diseases, which has been defined as a disease of the nasal and paranasal sinus mucosa present for greater than 3 months, with mucosal changes ranging from inflammatory thickening to nasal polyps [21]. Accumulations of activated eosinophils within tissues are the hallmark of this condition; however, the aetiology and pathophysiology of CRS are poorly understood [22, 23]. CRS is frequently seen in patients with asthma, and the two diseases share a number of pathological features, such as activation of T-helper type 2 (Th2)-like lymphocytes and eosinophils secreting IL-3, IL-5, IL-13, eotaxin and GM-SCF especially in an autocrine fashion. Thus, CRS is otherwise known as 'asthma of the upper airways' [22, 23]. The production of PGs by cultured cells from nasal polyps has previously been investigated [24, 25]. And the amount of PGs from polyp specimens has been quantified in a few reports [26, 27]. However, little is known with regard to the expression of terminal synthases responsible for PG production of patients with CRS despite the fact that PG synthase levels are often closely associated with the levels of the PGs they produce [28].

In the present study, we focused on the role of PGDS and PGES along with COX-1 and COX-2, the upstream regulators of PG synthesis, in the pathophysiology of CRS. We believe that the findings presented here provide new insight into the pathogenesis of CRS such as nasal polyp formation, and a basis for future therapeutic approaches aimed at synthase regulation to limit eosinophilic airway inflammation.

Materials and methods

Patients

Thirty-four Japanese patients (13–77 years old, mean 49.4 ± 18.4 , 21 males and 13 females) with CRS were studied [21]. Among these, 22 patients had nasal polyps endoscopically visualized in middle meatus (CRS-NP: 13–77 years old, mean 48.4 ± 19.0 , 13 males and nine females). The rest of the CRS patients ($n = 12$) had no visible polyps in middle meatus (CRS: 19–70 years old, mean 51.4 ± 17.9 , eight males and four females) [29]. All patients were resistant to medical treatment, including macrolide therapy, and thus had endonasal sinus surgery (ESS) [30]. Nine patients were asthmatic, and all of them

had nasal polyps. Among the nine asthmatic patients, two were thought to have aspirin sensitivity based on a clear-cut history of asthma attacks precipitated by non-steroidal anti-inflammatory drugs. None of the participants received systemic steroids for a period of at least 8 weeks before surgery, and none received pharmacotherapy for sinusitis, such as macrolide antibiotics or intranasal steroids, for a period of at least 3 weeks before surgery. Before surgery, serum total IgE levels (Pharmacia, Uppsala, Sweden), blood eosinophil counts and forced expiratory volume in 1 s (FEV₁; percent of predicted) were examined in each patient. A radiological assessment of the severity of sinusitis was also performed according to the Lund–MacKay system for each patient [31]. In addition, five non-CRS patients with normal uncinate process at inspection were enrolled (19–58 years old, mean 43.6 ± 18.6 , two males and three females): two suffering from blowout fracture, two from optic canal fracture and one from orbital tumour. Twelve of 39 subjects showed elevation of serum total IgE levels (> 170 IU/mL), and were thus considered as atopic. The clinical characteristics of groups of patients are shown in Table 1. Informed consent for participation in the study was obtained from each patient, and the study was approved by the Human Research Committee of the Okayama University Graduate School of Medicine and Dentistry.

Tissue sampling

ESS was performed under general anaesthesia. The nasal polyps from 22 CRS patients were excised and cut into small pieces. Half of the samples were immediately soaked in an RNeasyTM RNA stabilization reagent (Qiagen, Hilden, Germany) and stored at -30°C until use. The remaining samples were fixed with 4% paraformaldehyde and embedded in paraffin blocks. In 12 CRS patients not having nasal polyps and five non-CRS patients, uncinate process was excised and mucosae were removed from bone, and then immediately soaked in RNeasyTM.

Table 1. Subject characteristics

	CRS-NP	CRS	Control
Number	22	12	5
Sex (male : female)	13 : 9	8 : 4	2 : 3
Age (years)	48.4 ± 19.0	51.4 ± 17.9	43.6 ± 18.6
Age range (years)	13–77	19–70	19–58
Serum IgE (IU/mL)	315 ± 656	163 ± 262	116 ± 106
Atopic patients (serum IgE > 170 IU/mL)	8	3	1
Patients with bronchial asthma	9	0	0
Blood eosinophil ($\times 10^2/\mu\text{L}$)	4.55 ± 4.92	1.36 ± 0.96	2.16 ± 1.65
CT grading score	13.4 ± 6.4	5.8 ± 3.2	0 ± 0

CRS, chronic rhinosinusitis.

Immunohistochemistry

Immunohistochemical staining for h-PGDS, l-PGDS and m-PGES-1 was performed as previously described [32, 33]. In brief, paraffin-embedded polyp tissue was sectioned into 3 µm slices, deparaffinized, rehydrated and retrieved with trypsin (h-PGDS and l-PGDS) or pepsin (m-PGES-1) (Wako Pure Chemicals, Osaka, Japan). Endogenous peroxidase activity was quenched with 3% H₂O₂, and non-specific protein binding was blocked with 10% normal goat serum for 60 min. After this, the tissue sections were incubated with primary antibodies (anti-h-PGDS and anti-l-PGDS: rabbit poly; anti-m-PGES-1: guinea-pig poly) or control antibodies (rabbit IgG; Sigma, St Louis, MO, USA, or guinea-pig IgG; Inter-cell technologies Inc., Jupiter, FL, USA). A Vectastain Elite avidin-biotin-peroxidase kit (Vector Laboratories, Burlingame, CA, USA) with a diaminobenzidine substrate was used according to the manufacturer's instructions. In addition, sections were stained with haematoxylin/eosin to detect tissue eosinophils. In order to detect activated eosinophils, sections were reacted with anti-human eosinophil cationic protein/eosinophil protein X (ECP/EPX) (EG2; mouse IgG1) monoclonal antibody (Pharmacia), followed by goat anti-mouse immunoglobulin conjugated with a peroxidase-labelled amino acid polymer (Histofine Simple Stain MAX-PO (M), Nichirei Co., Tokyo, Japan), in accordance with the manufacturer's instructions. Positive cells were counted in two fields at high power (10 × 40) where the highest cellular infiltration was seen, from which the average number of positive cells was determined. In this study, we did not perform immunohistochemistry of uncinate process mucosae due to the small amount of tissue.

RT-PCR

Total cellular RNA was extracted using an RNeasyTM mini kit (Qiagen) in accordance with the manufacturer's instructions. The extracted material was treated with amplification grade deoxyribonuclease I (Sigma) for 15 min at room temperature. Reverse transcription of the samples to cDNA was performed using a first-strand cDNA synthesis kit (Toyobo, Osaka, Japan) in accordance with the manufacturer's instructions.

Real-time quantitative PCR assay was performed as described elsewhere [34]. In brief, the assay was performed using a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with QuantiTect SYBR Green PCR (Qiagen). The primers used for PCR had the following sequences and product sizes: h-PGDS, forward 5'-GAGAGCAGAAATTATTCGTTAC-3' and reverse 5'-CCAGAGTGTCCACAATAGCA-3' (248 bp); m-PGES-1, forward 5'-CTCTGCAGCACGCTGCTGG-3' and reverse 5'-GTAGGTCACGGAGCGGATGG-3' (339 bp); COX-1, forward 5'-TGC CCA GCT CCT GGC CCG CCG CTT-3' and reverse 5'-GTG CAT CAA CAC AGG CGC CTC TTC-3' (303 bp); COX-2, forward 5'-TGCCAGCACTTCA CGCATC-3' and reverse 5'-CTTTGACTGTGGGAGGATAC-3' (200 bp); and GAPDH, forward 5'-ACCACAGTCCATGC CATCAC-3' and reverse 5'-TCCACCACCCTGTTGCTGTA-3' (452 bp) [18, 35]. The 25 µL reaction mixture contained 300 nm each of forward and reverse primers, as well as QuantiTect SYBR Green PCR Master Mix (Qiagen), and 1 µL cDNA from a 20 µL volume of RT reaction mixture. Amplification was performed as follows: initial denaturation at 95 °C for 15 min, 40 cycles of denaturation at 94 °C for 15 s, annealing at 56 °C for 30 s and extension at 72 °C for 30 s. The amplified material was verified as single by examining the dissociation curve and ensuring it was of appropriate molecular weight by electrophoresis using 1.5% agarose gel, followed by sequencing. GAPDH from serially diluted plasmid DNA was amplified and used as a template, and a standard curve was plotted. The same method was used to quantify the genes of interest. The amount of GAPDH was used as an internal control. And each amount of the mRNA level was converted into numerical data by dividing their fluorescent signal by GAPDH level using the standard curve.

ward 5'-TGC CCA GCT CCT GGC CCG CCG CTT-3' and reverse 5'-GTG CAT CAA CAC AGG CGC CTC TTC-3' (303 bp); COX-2, forward 5'-TGCCAGCACTTCA CGCATC-3' and reverse 5'-CTTTGACTGTGGGAGGATAC-3' (200 bp); and GAPDH, forward 5'-ACCACAGTCCATGC CATCAC-3' and reverse 5'-TCCACCACCCTGTTGCTGTA-3' (452 bp) [18, 35]. The 25 µL reaction mixture contained 300 nm each of forward and reverse primers, as well as QuantiTect SYBR Green PCR Master Mix (Qiagen), and 1 µL cDNA from a 20 µL volume of RT reaction mixture. Amplification was performed as follows: initial denaturation at 95 °C for 15 min, 40 cycles of denaturation at 94 °C for 15 s, annealing at 56 °C for 30 s and extension at 72 °C for 30 s. The amplified material was verified as single by examining the dissociation curve and ensuring it was of appropriate molecular weight by electrophoresis using 1.5% agarose gel, followed by sequencing. GAPDH from serially diluted plasmid DNA was amplified and used as a template, and a standard curve was plotted. The same method was used to quantify the genes of interest. The amount of GAPDH was used as an internal control. And each amount of the mRNA level was converted into numerical data by dividing their fluorescent signal by GAPDH level using the standard curve.

Statistical analysis

Values were given as median and inter-quartile range (IQR). Non-parametric Mann-Whitney's *U*-test was used for comparing data between groups, and the Wilcoxon's signed-ranks test was used for analysis within the group. Correlation analysis was performed using Spearman's correlation coefficient by rank. A level of *P* < 0.05 was considered to be statistically significant. Statistical analyses were performed using StatViewTM software (version 4.5, Abacus Concepts Inc., Berkeley, CA, USA).

Results

Expression of h-PGDS, l-PGDS and m-PGES-1 in nasal polyps

Immunohistochemical staining revealed a marked expression of h-PGDS in the nasal polyps of patients with CRS. The expression was mainly seen in inflammatory cells and fibroblasts (Fig. 1a). On the other hand, minimal expression of l-PGDS was observed (Fig. 1b). Expression of m-PGES-1 was also noted. The expression was mainly seen on inflammatory cells (Fig. 1c). Thus, we sought to determine the relative amounts of h-PGDS and m-PGES-1 mRNA in nasal polyps using real-time PCR.

Gel image analysis provided the additional evidence that RT-PCR was working properly and that the enzymes were actually expressed in nasal polyps (Fig. 2). Real-time PCR revealed significantly greater amounts of

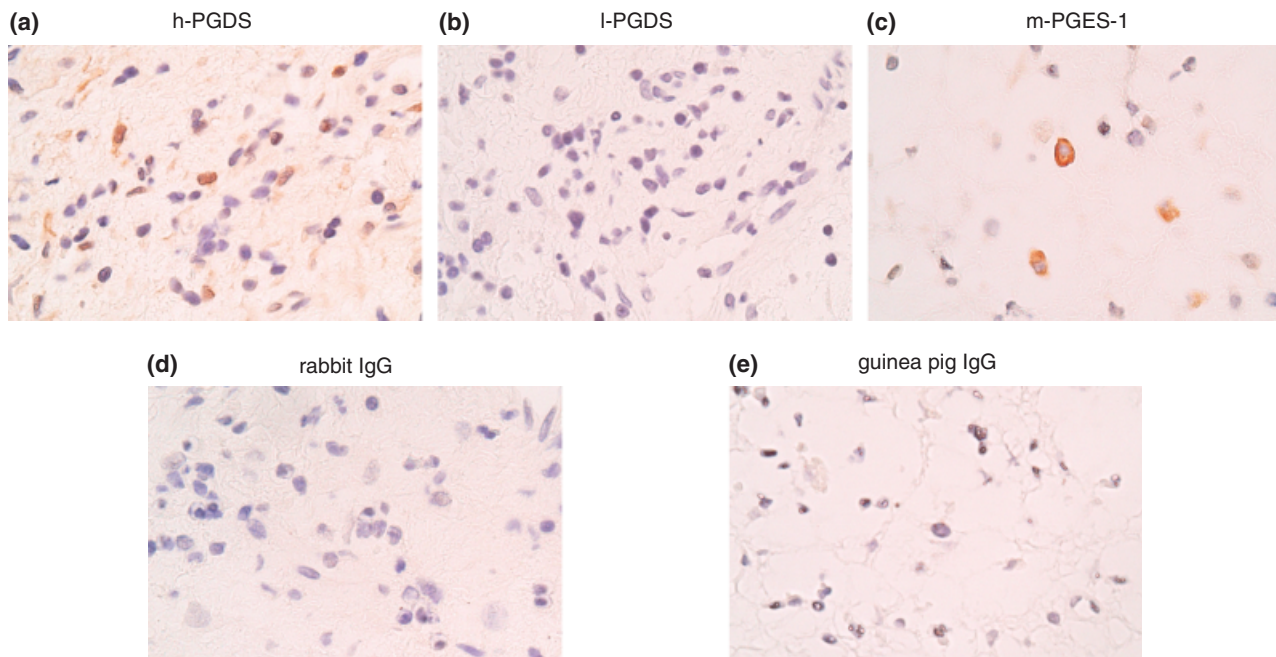


Fig. 1. Immunohistochemical staining of h-PGDS (a), l-PGDS (b) and m-PGES-1 (c) in nasal polyps. Sections of nasal polyps were reacted with rabbit polyclonal Ab against h-PGDS (a), rabbit polyclonal Ab against l-PGDS (b), guinea-pig polyclonal Ab against m-PGES-1 (c), rabbit IgG (d) or guinea-pig IgG (e), after which they were stained using a Vectastain Elite avidin-biotin-peroxidase kit with a diaminobenzidine substrate, as described in Materials and methods. Ab, antibody; h-PGDS, haemoipoietic-type prostaglandin D_2 synthase; l-PGDS, lipocalin-type-prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandinE2 synthase-1.

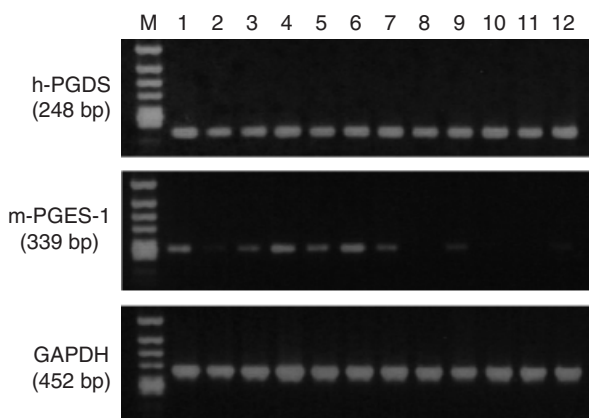


Fig. 2. Expression of mRNA specific for h-PGDS, m-PGES-1 and GAPDH in nasal polyps of CRS patients with (lanes 1–6) and without (lanes 7–12) asthma. mRNA was extracted from the nasal polyps, after which h-PGDS (248 bp), m-PGES-1 (339 bp) and GAPDH (452 bp) mRNA were detected by RT-PCR, as described in Materials and methods. M, molecular weight marker; h-PGDS, haemoipoietic-type prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandin E_2 synthase-1; CRS, chronic rhinosinusitis.

h-PGDS mRNA than m-PGES-1 mRNA within the polyps ($P < 0.0001$, Fig. 3).

Comparison of the relative amounts of h-PGDS and m-PGES-1 between nasal polyps and uncinate process mucosa

Levels of h-PGDS mRNA in nasal polyps were significantly higher than that in uncinate process mucosae ($P < 0.0001$, Fig. 4a). Conversely, levels of m-PGES-1 in nasal polyps were significantly lower than that in uncinate process mucosae ($P < 0.0001$, Fig. 4b). On the other hand, the presence or absence of CRS did not affect the levels of either h-PGDS ($P = 0.9161$) or m-PGES-1 ($P = 0.5982$) in uncinate process mucosae. In addition, a significant inverse correlation between h-PGDS and m-PGES-1 levels was observed in nasal polyps ($\rho = -0.557$, $P = 0.0101$) but not uncinate process mucosae ($\rho = 0.228$, $P = 0.3619$) (Fig. 5). GAPDH values between nasal polyps (median: 179 867, IQR: 184 582 molecules per 50 ng of RNA sample) and uncinate process mucosae (median: 215 860, IQR: 174 458 molecules per 50 ng of RNA sample) are not different ($P = 0.4789$).

Clinical significance of differences in the relative amounts of h-PGDS and m-PGES-1 in CRS

Among 34 CRS patients, significantly greater amounts of h-PGDS mRNA ($P < 0.0001$) and conversely lesser amounts of m-PGES-1 mRNA ($P < 0.0001$) in tissues were

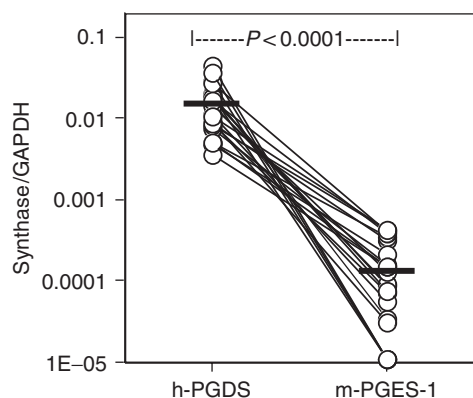


Fig. 3. Relative amounts of h-PGDS and m-PGES-1 mRNA in nasal polyps of patients with CRS ($n = 22$). h-PGDS and m-PGES-1 levels were estimated using real-time quantitative PCR. A log scale was used in the Y-axis. Each bar represents the median amount of mRNA for each protein. P -values were determined by Wilcoxon's signed-rank test. h-PGDS, haemopoietic-type prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandin E_2 synthase-1; CRS, chronic rhinosinusitis.

observed in those with nasal polyps compared with those without nasal polyps (Figs 6a and b). Significantly greater amounts of h-PGDS mRNA in tissues were observed in asthmatic patients (median: 0.0174, IQR: 0.0136) as compared with non-asthmatic patients (median: 0.0051, IQR: 0.0092, $P = 0.0036$). Significantly lesser amounts of m-PGES-1 were observed in asthmatic patients (median: 0.00003, IQR: 0.00009), compared with non-asthmatic patients (median: 0.00043, IQR: 0.00159, $P = 0.0006$). In detail, significantly lesser amounts of m-PGES-1 mRNA ($P = 0.0060$) but not h-PGDS ($P = 0.1166$) were observed in asthmatic patients compared with non-asth-

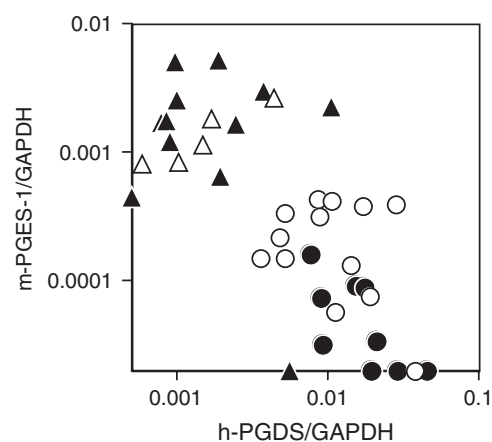


Fig. 5. Inverse relationship between h-PGDS and m-PGES-1 mRNA levels in sinonasal tissue from the following: $\triangle$, non-CRS patients; $\blacktriangle$, CRS patients without nasal polyps; $\circ$, CRS patients with nasal polyps without asthma; $\bullet$, CRS patients with nasal polyps and asthma. h-PGDS, haemopoietic-type prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandin E_2 synthase-1; CRS, chronic rhinosinusitis.

matic patients in the NP group (Figs 6c and d). On the other hand, the presence or absence of atopic status did not affect the amounts of h-PGDS or m-PGES-1 (data not shown). In addition, a significant positive correlation between h-PGDS levels and radiological severity of sinusitis was observed ($\rho = 0.573$, $P = 0.0010$, Fig. 7a), while a significant inverse correlation between m-PGES levels and the severity was observed ($\rho = -0.585$, $P = 0.0006$, Fig. 7b). Levels of either h-PGDS or m-PGES-1 mRNA were not correlated with blood eosinophil counts, serum total IgE levels or FEV₁ (percent of predicted) (data not shown).

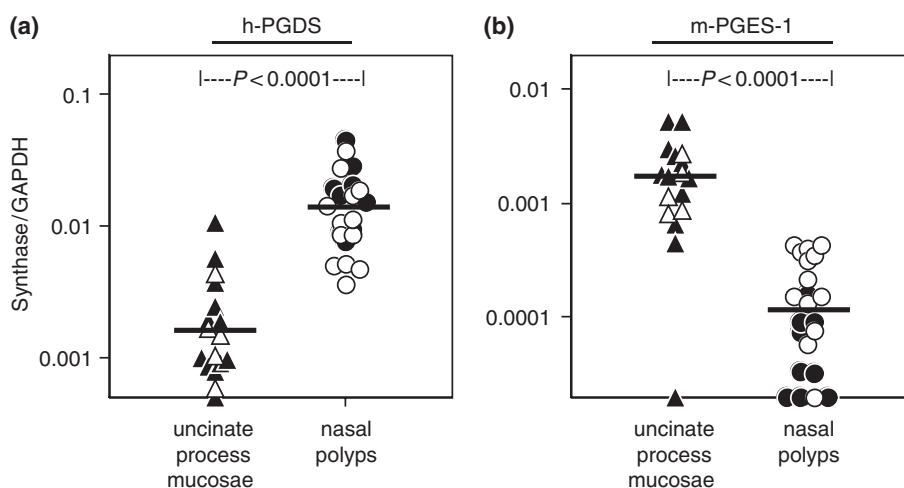


Fig. 4. Relative amounts of h-PGDS (a) and m-PGES-1 (b) mRNA in uncinata process mucosae ($n = 17$) and nasal polyps ($n = 22$). Uncinata process mucosae from non-CRS patients and CRS patients without nasal polyps are indicated as open and filled triangles, respectively. And nasal polyps from CRS patients with and without asthma are indicated as $\bullet$ and $\circ$, respectively. Each bar represents the median amount of mRNA for each protein. P -values were determined by Mann-Whitney's U -test. h-PGDS, haemopoietic-type prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandin E_2 synthase-1; CRS, chronic rhinosinusitis.

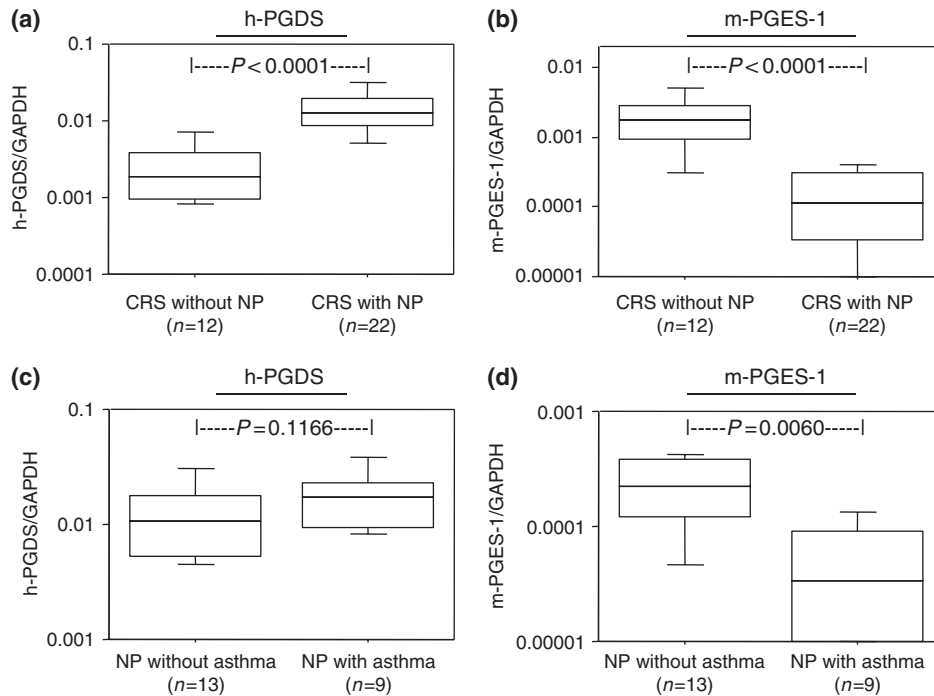


Fig. 6. Comparison of local h-PGDS (a, c) and m-PGES-1 (b, d) mRNA levels between CRS patients with and without nasal polyps (a, b) or between asthmatic and non-asthmatic patients in the NP group (c, d). The rectangle includes the range from 25th to 75th percentiles, the horizontal line indicates the median and the vertical line indicates the range from 10th to 90th percentiles. *P*-values were determined by Mann-Whitney's U-test. h-PGDS, haemopoietic-type prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandin E_2 synthase-1; CRS, chronic rhinosinusitis.

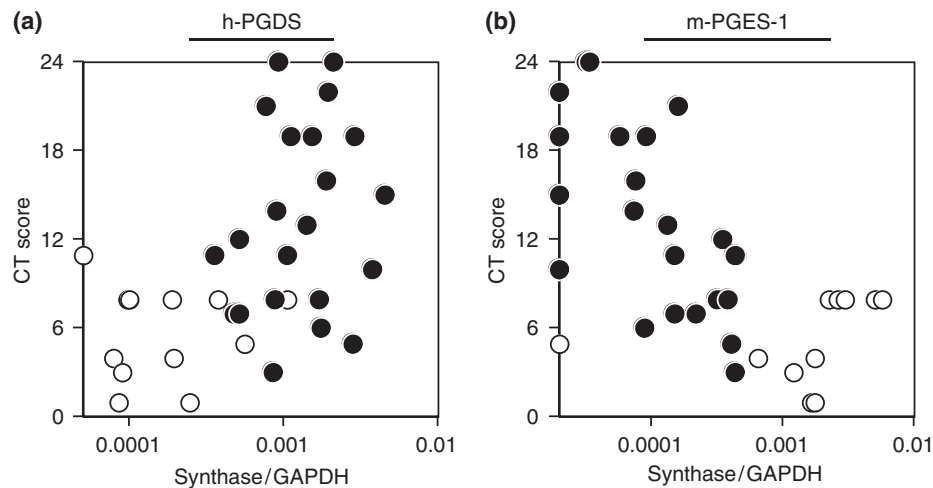


Fig. 7. Relationship between relative amounts of h-PGDS (a) and m-PGES-1 (b) mRNA in sinonasal tissues and radiological severity of sinusitis in 34 CRS patients. The radiological severity of sinusitis was scored according to the Lund-MacKay system. Twenty-two nasal polyps and 12 uncinate process mucosae are indicated as $\bullet$ and $\circ$, respectively. h-PGDS, haemopoietic-type prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandin E_2 synthase-1; CRS, chronic rhinosinusitis.

Association between PG synthase levels and degree of local eosinophil infiltration

We sought to determine whether h-PGDS and m-PGES-1 levels might be associated with local eosinophil accumulation. The degree of eosinophil infiltration into nasal

polyps, as determined by haematoxylin/eosin staining, was weakly correlated with h-PGDS ($\rho = 0.482$, $P = 0.0273$, Fig. 8a) and m-PGES-1 ($\rho = -0.401$, $P = 0.0635$, Fig. 8c) levels. Furthermore, the numbers of activated eosinophils, as determined by reactivity with EG2, were strongly and positively correlated with h-PGDS

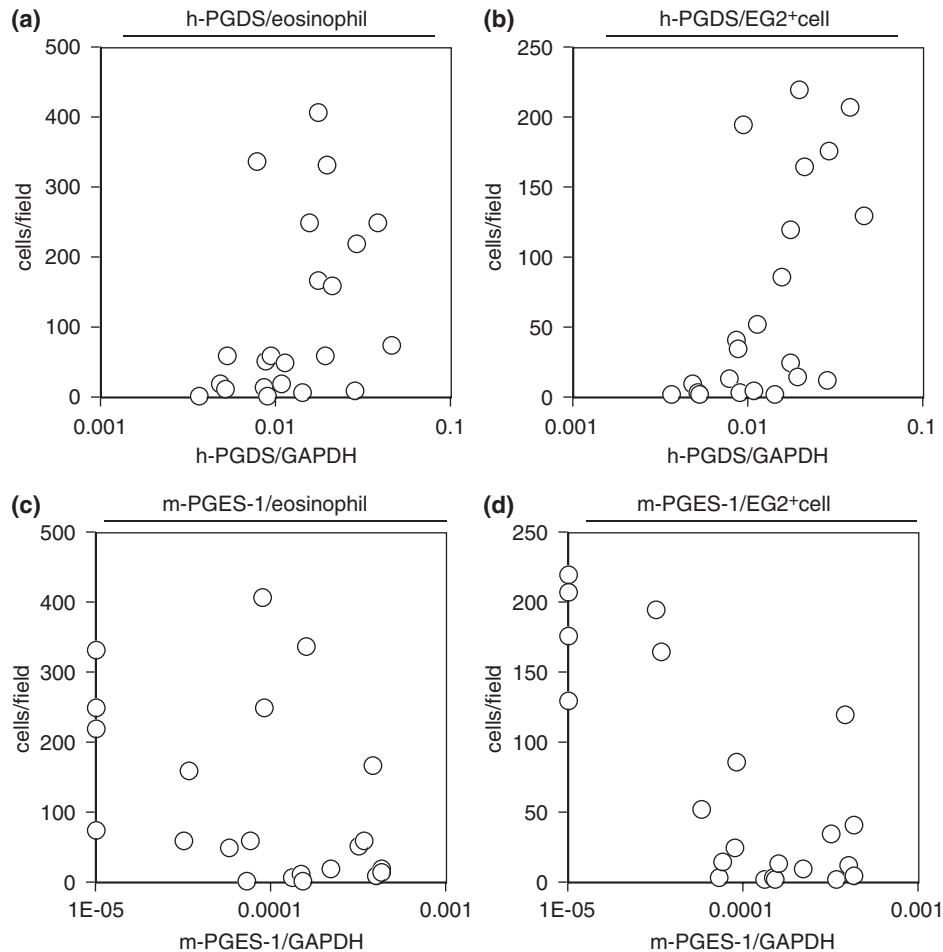


Fig. 8. Relationship between h-PGDS (a, b) and m-PGES-1 (c, d) expression in nasal polyps and number of infiltrating eosinophils (a, c) and EG2+ activated eosinophils (b, d). Positive cells were counted in two fields at high power (10×40) where the highest cellular infiltration was seen, from which the average number of positive cells was determined. h-PGDS, haemopoietic-type prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandin E_2 synthase-1.

levels ($\rho = 0.647$, $P = 0.0030$, Fig. 8b). Conversely, the numbers of EG2+ activated eosinophils were strongly and inversely correlated with m-PGES-1 levels ($\rho = -0.589$, $P = 0.0065$, Fig. 8d).

Relationship between COX and PG synthase levels

Finally, we examined the role of COXs, upstream regulators of the prostanoid pathway, in polyp formation and the severity of CRS. No difference in COX-1 expression was observed between uncinate process mucosae and nasal polyps ($P = 0.2569$, Fig. 9a). However, significantly higher levels of COX-2 expression were observed in nasal polyps as compared with uncinate process mucosa ($P = 0.0414$, Fig. 9b). Among 34 CRS patients, significantly lower levels of COX-2 ($P = 0.0443$) but not COX-1 ($P = 0.3190$) were observed in asthmatic patients (COX-1: median: 0.00060, IQR: 0.00063, COX-2: median: 0.00110, IQR: 0.00158), compared with non-asthmatic patients (COX-1:

median: 0.00091, IQR: 0.00096, COX-2: median: 0.00302, IQR: 0.00752). On the other hand, neither the levels of COX-1 ($P = 0.5173$) nor COX-2 ($P = 0.6948$) correlated with radiological severity of sinusitis. In addition, a trend in inverse correlation between the COX-2 levels and degree of infiltration into nasal polyps by both eosinophils ($P = 0.0691$) and EG2+ activated eosinophils ($P = 0.0555$) was observed. In contrast, the levels of COX-1 did not correlate with the degree of infiltration by eosinophils ($P = 0.7414$) or EG2+ cells ($P = 0.4005$).

Discussion

In the present study, we examined the expression and amount of terminal synthase enzymes that catalyse the synthesis of PGD₂ and PGE₂ in sinonasal tissues from patients with different conditions. It may be easier and more reliable to measure synthase, rather than PG, levels in nasal tissues as PGs can be easily inactivated by 15-hydroxyprostaglandin dehydrogenase within nasal

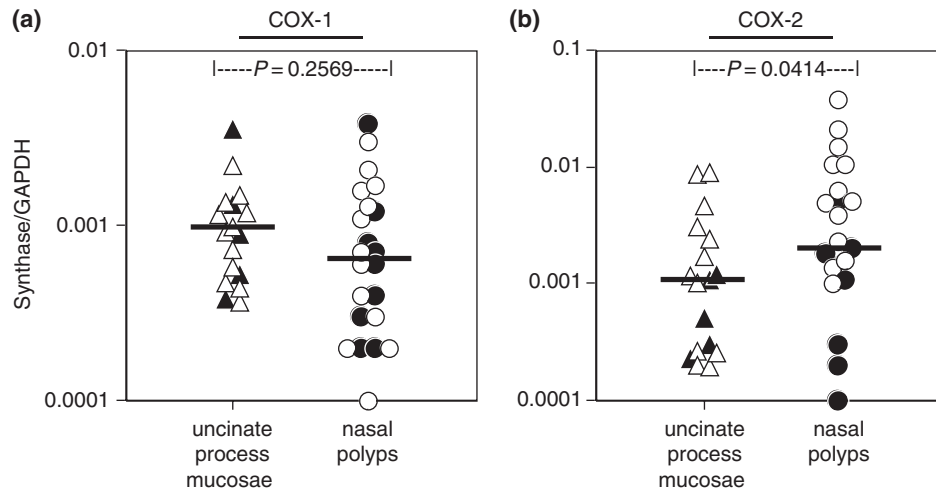


Fig. 9. Relative amounts of COX-1 (a) and COX-2 (b) mRNA in uncinata process mucosae ($n = 17$) and nasal polyps ($n = 22$). Uncinata process mucosae from non-CRS patients and CRS patients without nasal polyps are indicated as open and filled triangles, respectively. And nasal polyps from CRS patients with and without asthma are indicated as $\bullet$ and $\circ$, respectively. Each bar represents the median amount of mRNA for each protein. P -values were determined by Mann-Whitney's U -test. COX, cyclooxygenase; CRS, chronic rhinosinusitis.

mucosa [36]. To the best of our knowledge, this is the first report to demonstrate an inverse relationship between PGD2 and PGE2 terminal synthase levels and airway inflammation.

Significantly greater levels of h-PGDS mRNA and conversely lesser levels of m-PGES-1 mRNA were observed in the nasal polyps as compared with uncinata process mucosae. This result is consistent, in part, with the recent observation that PGE2 concentration is significantly decreased in nasal polyp tissue as compared with control sinonasal mucosae [28]. We believe that uncinata process mucosae are suitable as control for nasal polyps rather than inferior turbinate mucosae in order to investigate the pathophysiology of polyp formation as the polyps are usually developed from the osteomeatal complex including the process [18, 28, 37]. Together with the finding that the presence or absence of CRS did not affect the levels of either h-PGDS or m-PGES-1 in uncinata process mucosae, these results suggest that changes in the levels of PG synthase expression in osteomeatal complex play a critical role in the formation of nasal polyps. In fact, uncinata process mucosae from CRS patients without nasal polyps contain significantly lower levels of h-PGDS ($P < 0.0001$) and higher levels of m-PGES-1 ($P < 0.0001$) as compared with nasal polyps from CRS patients.

The level of h-PGDS mRNA was positively correlated with the degree of infiltration of the polyps by activated eosinophils and further correlated with radiological severity of sinusitis positively. It is known that marked eosinophil infiltration is associated with increased radiological severity of CRS [38]. This result is consistent with a prior observation that overexpression of PGDS promotes eosinophilic lung inflammation in a murine model of asthma [4]. And direct application of PGD2 induces

eosinophilia within airways [39]. Together with the finding that significantly greater amounts of h-PGDS mRNA in tissues were observed in asthmatic patients, our results suggest that h-PGDS displays a pro-inflammatory role in the pathophysiology of CRS. And increased expression of h-PGDS might enhance PGD2 production, thereby directly or indirectly inducing eosinophilic inflammation following interaction of PGD2 with DP and/or CRTH2 receptors on the surface of eosinophils [5, 40, 41]. Unlike h-PGDS, immunohistochemical staining could not detect l-PGDS in nasal polyps (Fig. 1b).

In contrast to h-PGDS, a negative correlation between levels of m-PGES-1 mRNA and degree of infiltration of nasal polyps by activated eosinophils was observed. Radiological severity of sinusitis also correlated inversely with m-PGES-1 levels in CRS patients. To date, it remains unclear whether PGE2 synthesis in airway disease has deleterious or beneficial effects [6–12]. The present findings are in agreement with a report by Pavord et al. [42], who found an inverse correlation between PGE2 concentrations and percentage of eosinophils within asthma-induced sputum. Together with the finding that significantly lesser amounts of m-PGES-1 mRNA in tissues were observed in asthmatic patients, our results suggest that m-PGES-1 displays an anti-inflammatory role in the pathophysiology of CRS. And in fact, inhalation of PGE2 attenuates allergen-induced increases in the relative proportions of sputum eosinophils and EG2+ cells [43]. PGE2 also suppresses the production of human airway smooth muscle cell-derived RANTES, a potent chemokine for eosinophils [44].

Furthermore, a significant inverse correlation between h-PGDS and m-PGES-1 expression was observed in the present study. We observed that the h-PGDS/m-PGES-1

ratio was strongly correlated ($P = 0.0009$) with the degree of infiltration of EG2+ activated eosinophils. These results suggest that an imbalance of h-PGDS and m-PGES-1 expression is critically associated with the pathophysiology of CRS. In fact, recent investigations suggest that the balance between PGD2 and PGE2 production contributes to the pathogenesis of several diseases [45, 46]. In atherosclerotic plaque formation, a switch from l-PGDS to m-PGES-1 expression by plaque macrophages is associated with cerebral ischaemia [45]. Another report suggests that increased secretion of PGDS by IL-1 β is completely inhibited by exposure to PGE2 [46]. Thus, decreased production of PGE2, combined with an increased production of PGD2, might synergistically augment eosinophilic airway inflammation.

Nasal polyps showed significantly higher levels of COX-2 but not COX-1 expression as compared with uncinate process mucosae. This is not surprising as up-regulation of COX-2 is usually found under inflammatory conditions [47]; however, this result differs from the previous reports showing the down-regulation of COX-2 in nasal polyps especially of aspirin-sensitive patients [28, 48]. This discrepancy may be due to different controls. Nasal mucosae were used as a control for nasal polyps in the previous reports, whereas we chose uncinate process mucosae as a control. Nasal polyps are mostly outgrown from uncinate process mucosae. We believe that uncinate process mucosae are more suitable than nasal mucosae as a control especially in order to investigate the pathogenesis of nasal polyp formation. On the other hand, another finding of ours that asthmatic patients showed reduced COX-2 levels as compared with non-asthmatics supports the previous reports [18, 28, 48], and suggests that the presence of asthma is closely associated with a selective impairment of COX-2 expression in CRS. In addition, PG terminal synthases rather than COXs appear to display a crucial role in the pathogenesis of CRS as the levels of h-PGDS and m-PGES-1 were more significantly correlated with local eosinophils and radiological severity than the levels of COX.

COX-2 levels within uncinate process mucosae were not significantly correlated with m-PGES-1 levels ($\rho = 0.400$, $P = 0.1100$). However, COX-2 levels within nasal polyps were significantly and positively correlated with m-PGES-1 levels ($\rho = 0.548$, $P = 0.0122$). This result seen in the inflamed polyp tissue is reasonable as m-PGES-1 and COX-2 are co-localized upon stimulation [15, 49]. And it is further suggested that impaired COX-2 generation, together with decreased m-PGES-1 expression, might strongly limit the production of PGE2 in nasal polyps, especially in asthmatic patients. A marked deficiency in PGE2 production might augment eosinophilic airway inflammation [9–12].

In conclusion, the present study shows an inverse relationship between h-PGDS and m-PGES-1 expression

and pathophysiology in CRS. These results suggest that pathways involved in PGD2 and PGE2 generation are potential therapeutic targets for the treatment of CRS. In particular, asthmatic patients demonstrate greater abnormalities of COX-2/h-PGDS/m-PGES-1 expression. Thus the therapeutic potential of ensuring adequate COX-2/h-PGDS/m-PGES-1 signalling might be extended to the treatment of asthma, as CRS is also known as 'asthma of the upper airway' due to similarities in the pathogenesis of the two conditions [23].

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