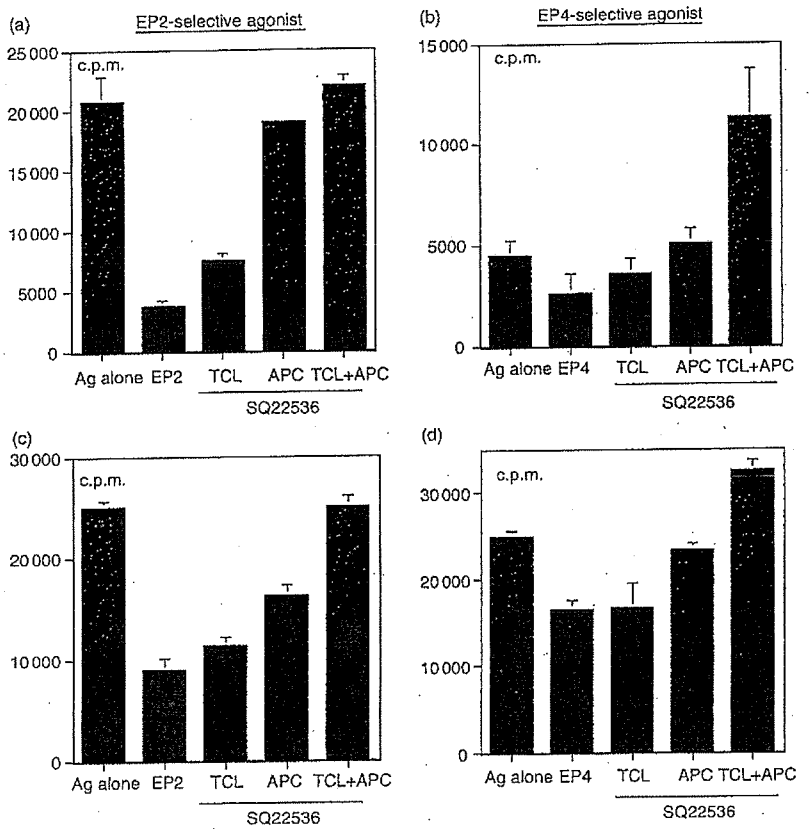

Figure 5. Reversal of EP2/EP4-induced inhibition of amtigen-specific T-cell responses with an adenylate cyclase inhibitor. Cry j 1-specific (a,b) and PPD-specific (c,d) TCL alone, APC alone or both TCL and APC were pretreated with SQ22536 at 37° for 1 hr. Following incubation, the cells were washed with culture medium three times, after which they were mixed and cultured with the respective antigen in the presence of an EP2 receptor agonist (a,c) or an EP4 receptor agonist (b,d) at a concentration of 0·2 μM for 72 hr. Typical proliferative responses are shown in mean c.p.m. ± SD from triplicate cultures. The baseline proliferations in the absence of Cry j 1 were 69 ± 5, 56 ± 14 and 66 ± 38 for OG-J1 (a), YJ-15 (b) and YP-11 (c,d), respectively. Data are representative of at least three separate experiments.

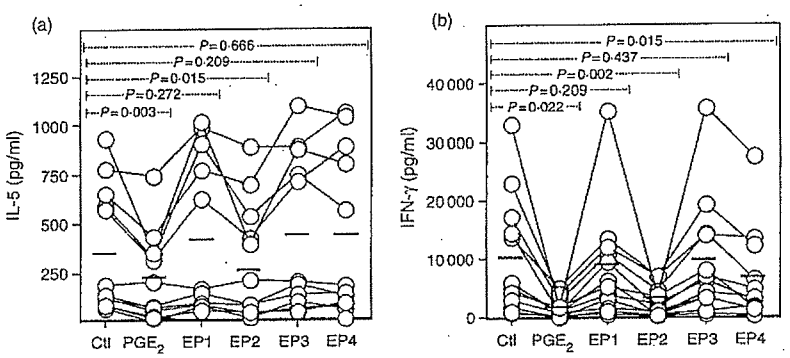

Figure 6. PGE_2/EP receptor-selective agonists-mediated inhibition of antigen-specific cytokine production by PBMCs. PBMCs from 12 patients with Japanese cedar pollinosis were cultured with 10 μg/ml of Cry j 1 (a), or 2 μg/ml of PPD (b), in the presence of PGE_2 and an EP receptor agonist or control buffer, each at a concentration of 1 μM for 72 hr. Following incubation, supernatant was collected and concentrations of IL-5 (a) and IFN-γ (b) were determined in each sample using ELISA. *P*-values were obtained using Wilcoxon's signed-rank test. The baseline productions of IL-5 and IFN-γ in the absence of antigen were 0 ± 0 and 0 ± 0 pg/ml, respectively.

Effect of PGE_2 and EP receptor-selective agonists on Cry j 1- and PPD-specific PBMC responses

Next, we investigated the effect of PGE_2 on Cry j 1- and PPD-specific T-cell responses in PBMCs. In patients with Japanese cedar pollinosis 10^{-6} M PGE_2 significantly inhibited IL-5 and IFN-γ production by PBMCs in response to stimulation with Cry j 1 and PPD, respectively (Fig. 6). Among four EP receptor-selective agonists, only the EP2 receptor agonist significantly inhibited Cry j 1-specific IL-5 production (Fig. 6a). On the other hand, both EP2 and EP4 receptor agonists significantly inhibited PPD-specific IFN-γ production (Fig. 6b). The EP1 and EP3 receptor agonists had no effects on antigen-specific

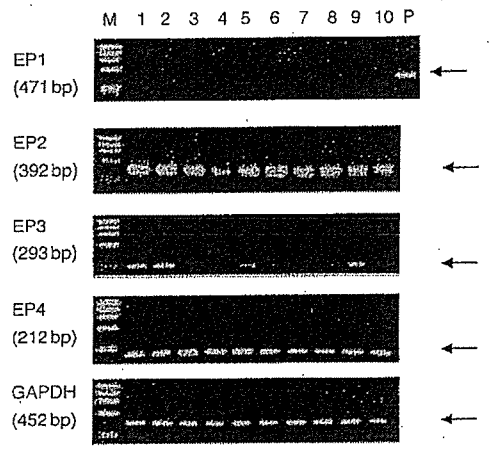

Figure 7. Expression of EP receptors by human TCLs. Messenger RNA was extracted from five Cry j 1-specific TCLs (lanes 1–5) and five PPD-specific TCLs (lanes 6–10), after which levels of EP1, EP2, EP3, EP4 and GAPDH were detected by RT-PCR as described in the Materials and methods section. M, molecular marker; P, positive control (genomic DNA).

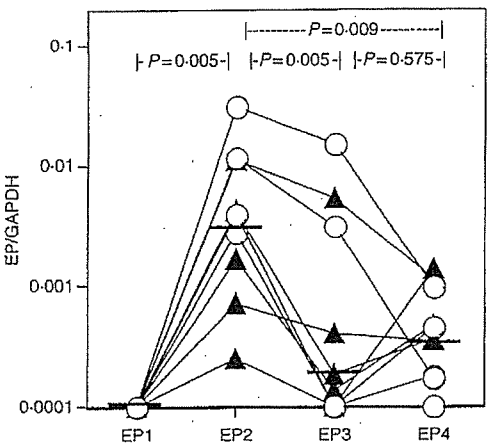

Figure 8. Comparison of EP receptor expression among TCLs. The expression levels of four EP receptors were determined in five Cry j 1-specific TCLs (O) and five PPD-specific TCLs (▲) using real-time RT-PCR. Each bar represents the median expression level of each messenger. *P*-values were obtained using Wilcoxon's signed-rank test.

cytokine production by PBMCs. PBMCs from control subjects not sensitized with Japanese cedar pollen did not produce IL-5 in response to Cry j 1 (data not shown). However, those produced a significant amount of IFN-γ in response to PPD (mean 5953 pg/ml). Only the EP2 receptor agonist significantly inhibited PPD-specific IFN-γ production (mean 2558 pg/ml: $P = 0{\cdot}028$).

Expression of the four EP receptors on Cry j 1- and PPD-specific TCLs

Finally, messenger RNA expression of the four EP receptors was examined in five Cry j 1- and five PPD-specific TCLs by RT-PCR. EP1 expression was almost undetectable in all TCLs. However, EP2 and EP4 mRNA were clearly detected in all TCLs. EP3 expression varied among the cells (Fig. 7). Relative expression levels of the four EP receptors were not observed to differ among Cry j 1- and PPD-specific TCLs (EP2: $P = 0{\cdot}148$, EP3: $P = 0{\cdot}917$, EP4: $P = 0{\cdot}117$, using Mann–Whitney's *U*-test). However, significantly increased expression of the EP2 receptor, compared to the other subtypes, was observed in both types of TCLs (Fig. 8).

Discussion

In the present study, we examined the effect of PGE_2 on antigen-specific human T-cell responses. PGE_2 dose-dependently inhibited Cry j 1- and PPD-induced T-cell responses in cultured TCLs and freshly isolated PBMCs. In addition, EP2 and EP4 receptor agonists also inhibited these antigen-specific responses, and the inhibition was restored by the addition of an adenylate cyclase inhibitor. These results suggest that PGE_2 suppresses both Th1- and Th2-polarized antigen-specific human T-cell responses via a cAMP-dependent EP2 and/or EP4-mediated pathway.

Early reports showed inhibition of IFN-γ and IL-2 production from $CD4^+$ T cells by PGE_2, however, no changes in IL-4 production were observed.[11,12] More recent reports, however, have revealed that the effects of PGE_2 on cytokine production are highly influenced by external stimuli.[15–18] For example, PGE_2 has been observed to inhibit IL-4 gene expression in anti-CD3- plus anti-CD28-activated T cells, however, not when the cells are stimulated with phorbol 12-myristate 13-acetate plus a calcium ionophore.[16] In addition, Dooper *et al.* recently reported that PGE_2 inhibited concanavalin A-stimulated IFN-γ but not IL-2 production by PBMC.[18] These results suggest that the effect of PGE_2 on cytokine production may differ depending on whether T cells are stimulated with MHC-mediated antigen-specific signals or nominal T-cell receptor signals. Since most of what is known about PGE_2 has been demonstrated in the presence of nominal T-cell receptor signals, we sought to determine the role of PGE_2 alone in antigen-specific human T-cell responses. To the best of our knowledge, this is the first report to demonstrate the effect of PGE_2 and PGE_2 receptor agonists on antigen-specific human T-cell responses.

In this study, antigen-induced production of IL-4 from Cry j 1-specific TCLs, and IFN-γ from PPD-specific TCLs, was suppressed by treatment with PGE_2. This result differs from the results of two previous studies.[11,12] However, our finding is consistent with a recent report by He and Stuart indicating that PGE_2 inhibits the production of IL-2 and IFN-γ, as well as IL-4, IL-5, and IL-10, by

human $CD4^+$ T-cell clones stimulated with anti-CD3 monoclonal antibody.[27] The baseline production of IL-4 by Cry j 1-specific TCLs and of IFN-γ by PPD-specific TCLs in the absence of antigen were 0 ± 0 and 203 ± 314 pg/ml, respectively, and no additional proliferation or cytokine production over background was observed with ovalbumin, the irrelevant antigen. In addition, endotoxin contamination was considered to be negligible because the result of an Endospec™ ES test was negative. These results suggest that the cellular responses presented here represent bona fide antigen-specific restimulation of rested T cells.

It is unclear why PGE_2 might inhibit the production of both IL-4 and IFN-γ by TCLs. It is possible that T cells stimulated by specific antigens are more sensitive to inhibition by PGE_2 than when stimulated by other stimulants, including mitogens and antibodies against surface proteins. PGE_2 has been reported to selectively inhibit human $CD4^+$ T cells secreting small amounts of IL-2 and IL-4. Although we have not yet determined the amount of IL-2 produced by the TCLs examined in this experiment, it does not appear that low levels of cytokine production resulted in an increased response to PGE_2 in the present study. This was supported by the fact that Cry j 1-specific TCLs almost failed to produce IL-4 in the presence of 1 μM PGE_2 (96·72 ± 0·87% inhibition), regardless of the initial level of IL-4 production noted following antigen stimulation (from 53·0 to 685·0 pg/ml, mean 322·2 ± 228·3 pg/ml. Fig. 2). Again, this inconsistency might be because of differences in exposure of the TCLs to external stimuli in the two studies.

PGE_2 also inhibited the production of Cry j 1-induced IL-5 production, as well as PPD-induced IFN-γ production, from PBMCs, respectively. It is well known that PBMCs from patients with Japanese cedar pollinosis produce IL-5 in response to Cry j 1 but that PBMCs from asymptomatice subjects do not.[28] Thus we used IL-5 as a marker of the Th2 response in PBMCs because, unlike Cry j 1-specific TCLs, IL-4 production by PBMCs in response to Cry j 1 is marginal.[23] Together with the result that the contamination of endotoxin in Cry j 1 is negligible by endospec assay (SeiKagaku Kogyo Corporation, Tokyo, Japan), it is suggested that IL-5 is indeed produced by PBMCs from the patients in an antigen-specific manner. Since PGE_2-induced cellular responses are known to differ between cultured T cells and freshly isolated PBMCs,[11] the present results suggest that the inhibitory effect of PGE_2 on antigen-specific cytokine production was also seen in a more physiological situation. In addition, the effect of PGE_2 on IL-5 production by human T cells remains controversial.[11–14] Our results match a report by Snijdewint *et al.* demonstrating that IL-5 production by PBMCs stimulated with anti-CD2 plus anti-CD28 monoclonal antibodies is significantly inhibited by the addition of 1 nM PGE_2. [11]

A few reports have demonstrated the role of EP receptor isoforms in immune responses influenced by PGE_2.[29–31] Nataraj *et al.* observed that the EP2 receptor plays a dominant role in PGE_2-mediated inhibition of mixed lymphocyte reactions in mice.[29] Walker and Rotondo recently reported that suppressive effects of PGE_2 on IL-12 and IL-18-induced IFN-γ synthesis by natural killer cells are mediated via EP2 receptors.[31] Among four EP receptor-selective agonists, we observed the EP2 receptor agonist to have the greatest inhibitory effect on both Cry j 1-specific and PPD-specific cellular responses in TCLs and PBMCs. The EP4 receptor agonist also had an inhibitory effect; however, this effect was weak compared with that of the EP2 receptor agonist and did not result in inhibition of Cry j 1-specific IL-5 production by PBMCs. EP1 and EP3 receptor agonists, on the other hand, had no effect. Since EP2 and EP4, but not EP1 or EP3, are Gs-coupled receptors, our results suggest that the inhibitory effect of PGE_2 on antigen-specific cellular responses might be mediated by activation of the Gs protein through binding of EP2 and/or EP4.[21] It seems to be a bimodal distribution: high and low responders in the 12 patitents with allergic rhinitis especially for IL-5 production. However, the EP2 receptor agonist significantly inhibited Cry j 1-specific IL-5 production by PBMC from both high ($n = 5$: $P = 0·043$) and low ($n = 7$: $P = 0·028$) responders.

Little is known about the expression of EP receptors on human T cells. We detected messenger RNA expression of EP2, EP3 and EP4, but not EP1, receptors in both Cry j 1- and PPD-specific TCLs. This result differs from the observations of Nataraj *et al.* who observed messenger RNA expression of EP1, EP2 and EP4, but not EP3, receptors on splenic T cells in mice.[29] It is possible that T-cell expression of the four subtypes of EP receptors varies among different species. In addition, more research is needed to determine the expression of these receptors on various T-cell subsets, such as naive and memory T cells. However, the fact that we observed a predominance of EP2 receptors on TCLs is probably related to the marked inhibitory effect of the EP2 receptor agonist on antigen-specific cellular responses. This predominance may explain why signals through EP3, which can inhibit adenylate cyclase, had little effect on EP2-mediated suppression (Fig. 2).

Pretreatment of both TCLs and APCs with SQ22536 completely reversed the inhibitory effects of EP2 and EP4 receptor agonists on antigen-specific cellular responses. This result probably relates to the fact that EP2 and EP4 both stimulate adenylate cyclase.[21] These results are consistent with a previous report indicating that the cAMP-dependent signalling pathway inhibits the production of Th1- and Th2-related cytokines.[17] In addition, a recent report has demonstrated that cAMP inhibits T-cell activation by triggering PKA type I.[32] This is supported by our

finding that pretreatment of TCLs and/or APCs with Rp-8-bromo-cAMP-phosphorothiate, a PKA type I antagonist, partially reversed the inhibitory effects of EP2 and EP4 receptor agonists on Cry j 1-specific and PPD-specific T-cell responses.

PGE_2 seems to influence the antigen-specific cellular responses of both T cells and APCs because reversal of inhibition was seen by pretreatment of both with SQ22536. Pretreatment of APCs with SQ22536 caused a more marked reversal of the PGE_2-mediated response than pretreatment of TCLs, suggesting that PGE_2 may have a greater effect on the antigen-specific cellular responses of APCs. PGE_2 is known to affect various other APC functions, such as expression of MHC class II molecules and cytokine production of tumour necrosis factor-α and IL-12.[33,34] In addition, we have recently reported that PGE_2 inhibits the expression of several costimulatory molecules through EP2/EP4 in human monocytes.[30]

In conclusion, we have provided *in vitro* evidence that PGE_2 inhibits both Th2- and Th1-polarized antigen-specific human T-cell responses. Stimulation of EP2 and EP4 and subsequent activation of adenylate cyclase and PKA type I might mediate these effects. These observations might provide a basis for future therapeutic approaches in the management of diseases, such as type I allergy and autoimmune diseases, in which antigen-specific T-cell responses are involved.

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ORIGINAL PAPER

Role of prostaglandin D_2 and E_2 terminal synthases in chronic rhinosinusitis

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Clinical and Experimental Allergy

Summary

Background Prostaglandin (PG)D_2 and E_2, two major cyclooxygenase (COX) products, are generated by PGD_2 synthase (PGDS) and PGE_2 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.

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Introduction

Prostaglandins (PGs) are lipid mediators that regulate immune function [1]. Among these, PGD_2 and PGE_2 are thought to mediate airway inflammation [2]. PGD_2 mediates a variety of biological activities, including vasodilation and bronchoconstriction [3]. Overproduction of PGD_2 induces eosinophilic lung inflammation, and deletion of DP, a PGD_2 receptor, reduces eosinophilic inflammation in murine models of asthma [4, 5]. PGE_2 has a pro-inflammatory role by enhancing eosinophil survival and mucin gene expression [6–8]. However, PGE_2 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 PGE_2 are deleterious or beneficial in the pathogenesis of airway inflammation.

PGD_2 and PGE_2 are derived from arachidonic acid (AA). AA is mobilized from membrane phospholipids by phospholipases and is converted to PGH_2 by cyclooxygenase (COX) enzymes. PGH_2 is acted upon by PGD_2 synthase (PGDS) and PGE_2 synthase (PGES) to produce PGD_2 and PGE_2, 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 (FEV_1; 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 RNAlater™ 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 RNAlater™.

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 ($\times 10^2/\mu L$)	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% H_2O_2, 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 RNeasy™ 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′-TGCCCAGCACTTCA 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 StatView™ 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

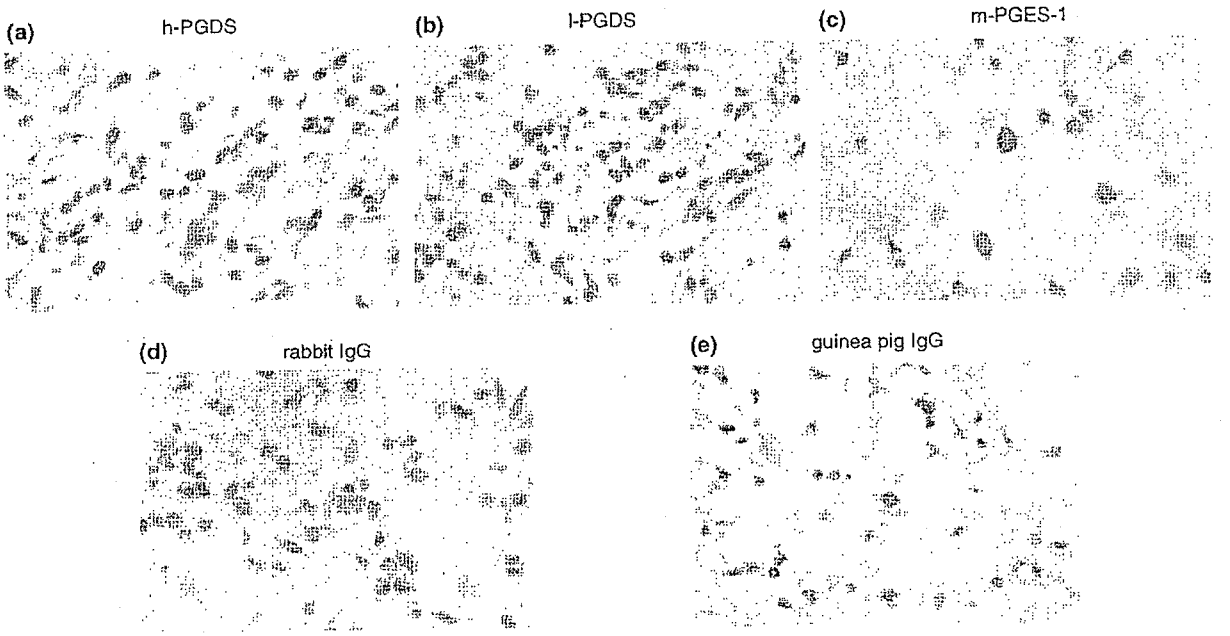

Fig. 1. Immunohistochemical staining of h-PGDS (a), l-PGDS (b) and m-PGES-1 (c) in nasal polyps. Sections of nasal polyps were reacted with rabbit polyclonal Ab against h-PGDS (a), rabbit polyclonal Ab against l-PGDS (b), guinea-pig polyclonal Ab against m-PGES-1 (c), rabbit IgG (d) or guinea-pig IgG (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, haemopoietic-type prostaglandin D_2 synthase; l-PGDS, lipocalin-type-prostaglandin D_2 synthase; m-PGES-1, microsomal prostaglandinE2 synthase-1.

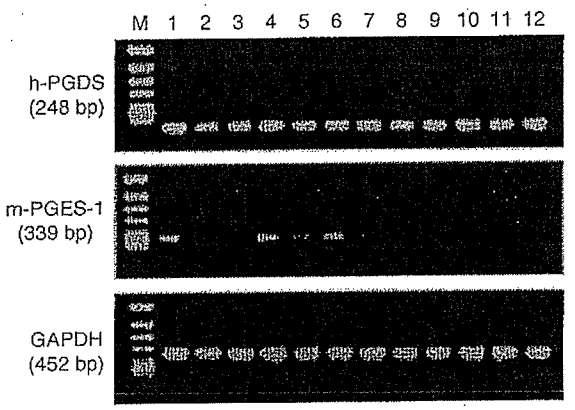

Fig. 2. Expression of mRNA specific for h-PGDS, m-PGES-1 and GAPDH in nasal polyps of CRS patients with (lanes 1–6) and without (lanes 7–12) asthma. mRNA was extracted from the nasal polyps, after which h-PGDS (248 bp), m-PGES-1 (339 bp) and GAPDH (452 bp) mRNA were detected by RT-PCR, as described in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

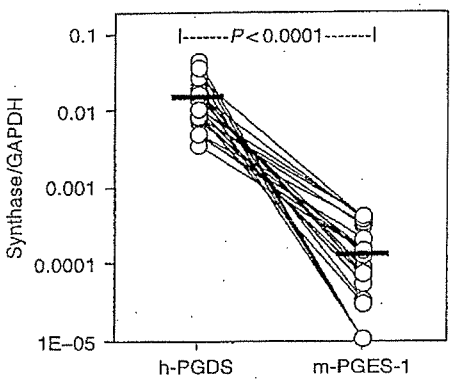

Fig. 3. Relative amounts of h-PGDS and m-PGES-1 mRNA in nasal polyps of patients with CRS ($n = 22$). h-PGDS and m-PGES-1 levels were estimated using real-time quantitative PCR. A log scale was used in the *Y*-axis. Each bar represents the median amount of mRNA for each protein. *P*-values were determined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

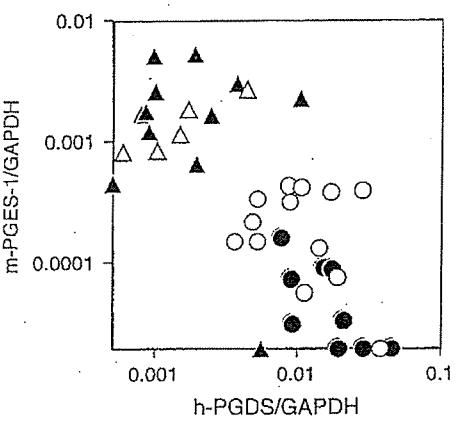

Fig. 5. Inverse relationship between h-PGDS and m-PGES-1 mRNA levels in sinonasal tissue from the following: △, non-CRS patients; ▲, CRS patients without nasal polyps; ○, CRS patients with nasal polyps without asthma; ●, CRS patients with nasal polyps and asthma. h-PGDS, haemopoietic-type prostaglandin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

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-asthmatic 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 FEV_1 (percent of predicted) (data not shown).

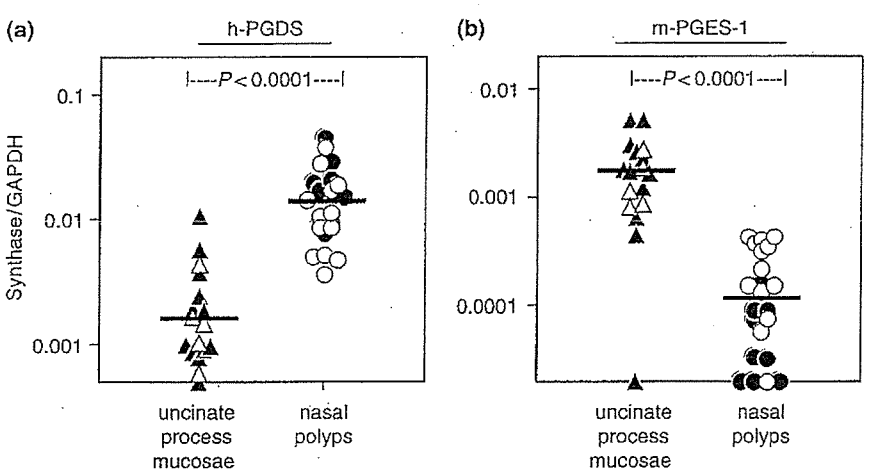

Fig. 4. Relative amounts of h-PGDS (a) and m-PGES-1 (b) mRNA in uncinate process mucosae ($n = 17$) and nasal polyps ($n = 22$). Uncinate process mucosae from non-CRS patients and CRS patients without nasal polyps are indicated as open and filled triangles, respectively. And nasal polyps from CRS patients with and without asthma are indicated as • and ○, 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.

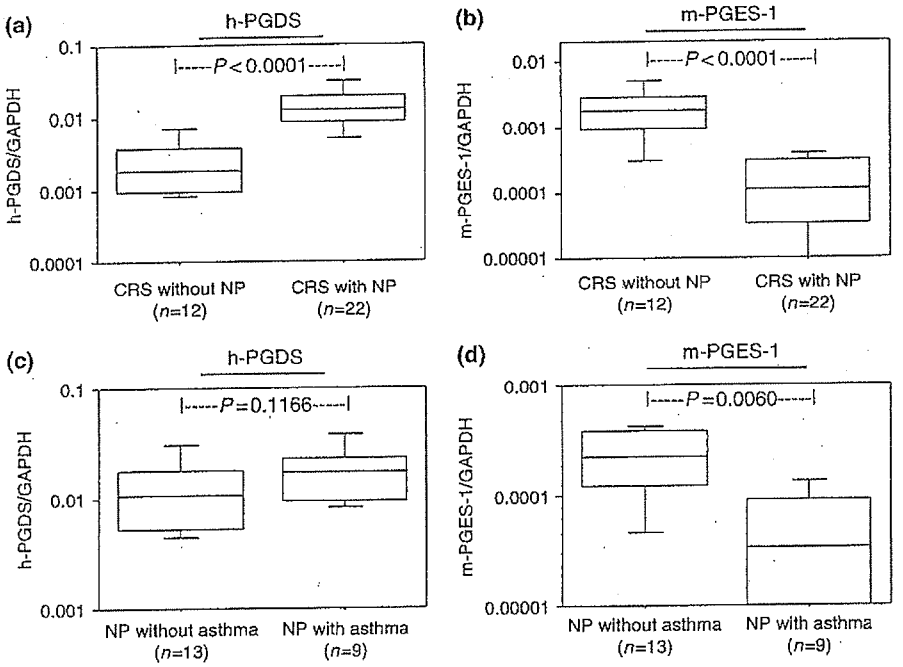

Fig. 6. Comparison of local h-PGDS (a, c) and m-PGES-1 (b, d) mRNA levels between CRS patients with and without nasal polyps (a, b) or between asthmatic and non-asthmatic patients in the NP group (c, d). The rectangle includes the range from 25th to 75th percentiles, the horizontal line indicates the 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.

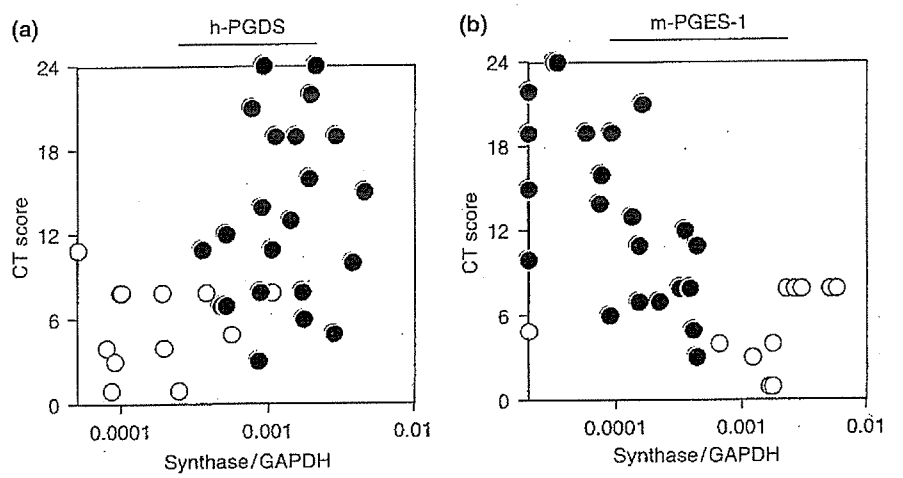

Fig. 7. Relationship between relative amounts of h-PGDS (a) and m-PGES-1 (b) mRNA in sinonasal tissues and radiological severity of sinusitis in 34 CRS patients. The radiological severity of sinusitis was scored according to the Lund–MacKay system. Twenty-two nasal polyps and 12 uncinate process mucosae are indicated as • and o, 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

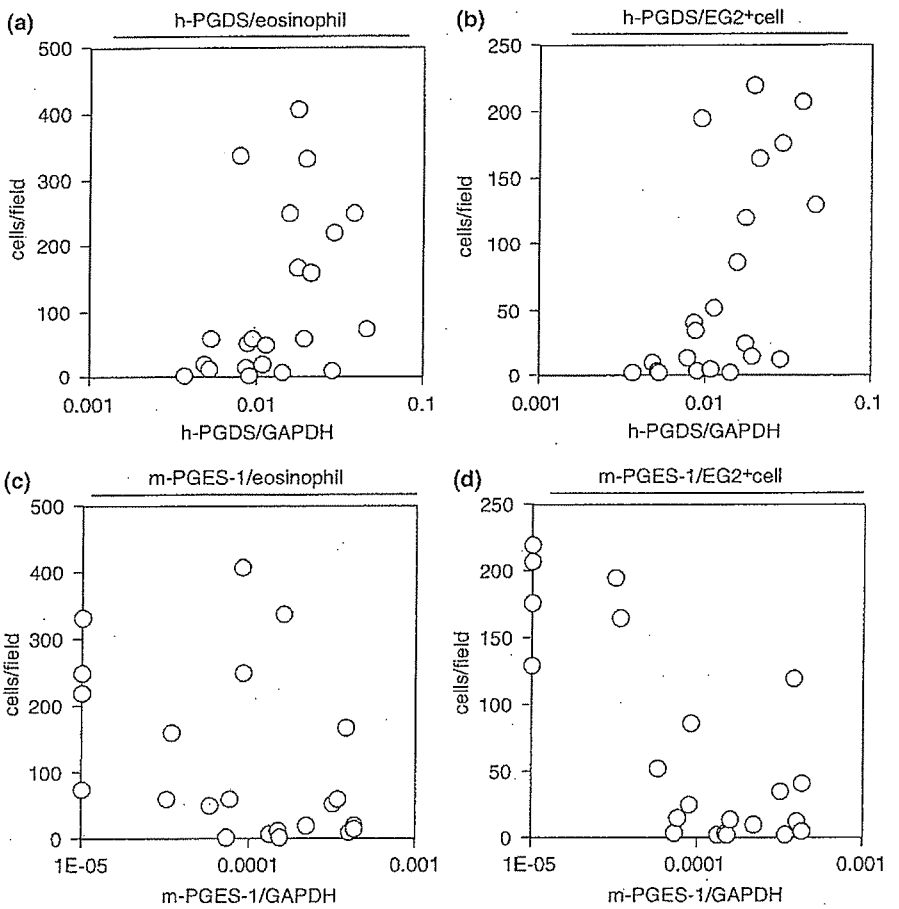

Fig. 8. Relationship between h-PGDS (a, b) and m-PGES-1 (c, d) expression in nasal polyps and number of infiltrating eosinophils (a, c) and EG2+ activated eosinophils (b, d). Positive cells were counted in two fields at high power (10 × 40) where the highest cellular infiltration was seen, from which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

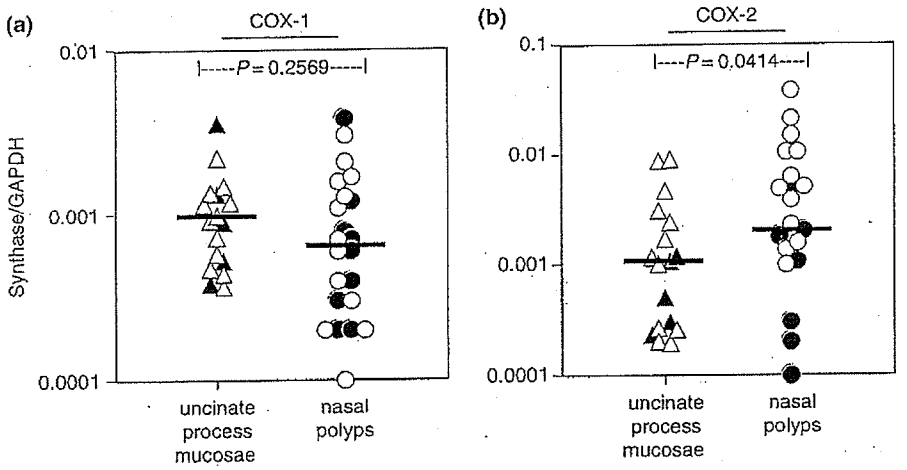

Fig. 9. Relative amounts of COX-1 (a) and COX-2 (b) mRNA in uncinate process mucosae ($n = 17$) and nasal polyps ($n = 22$). Uncinate process mucosae from non-CRS patients and CRS patients without nasal polyps are indicated as open and filled triangles, respectively. And nasal polyps from CRS patients with and without asthma are indicated as • and ○, 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 uncinate 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 uncinate 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 uncinate 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, uncinate 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-1β 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 asthamtic 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 eosinophila 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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研究報告書

細胞内シグナルを利用したスギ花粉症治療可能性

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研究要旨
アレルギー性鼻炎粘膜では、抗原が鼻腔内に進入し肥満細胞上のIgEと架橋するとシグナルを発生して、ヒスタミンなどのケミカルメディエイターを放出する。その代表的なシグナルとしてSykが存在する。Sykはアレルギー性鼻炎において極めて重要な因子であり、血球系のみならず鼻粘膜線維芽細胞にも発現し機能していることを我々は見出してきた。今回鼻粘膜におけるSyk発現細胞を検討したところ、鼻粘膜上皮の好酸球に強くかつ多く発現されていることが判明した。BLyS (B cell stimulator)は、B細胞刺激因子であるが、その役割は十分にはわかっていない。本研究では、抗CD40抗体が低濃度ではBLySはIgEクラススイッチを有意に増強し、BLyS中和抗体前処理によりIL-4と抗CD40抗体によって誘導されるIgE産生が減少した。下甲介粘膜由来線維芽細胞ではTLR3, LTR4, TLR9の発現が多く, Poly ICとLPS刺激によりBLySが発現した。これらのことからBLySの制御もアレルギー性鼻炎の標的分子になりえることが判明した。

A. 研究目的

アレルギー性鼻炎の治療は、確実にIgE-dependentな疾患である。そのためIgE産生（クラススイッチ）を抑制できる手段は、ほとんどがアレルギー性鼻炎の治療手段になりえると考えられている。BLyS (B cell stimulator)は、シェーグレン症候群・SLE・リウマチ性関節炎などの自己免疫疾患の病因の一つとして知られているが, CD40非依存性に免疫グロブリン(Ig)のクラススイッチを誘導することも報告されている。一方で我々は、鼻粘膜においてIgEクラススイッチが誘導されていること、鼻粘膜における線維芽細胞がアレルギー性鼻炎の発症、病態に大きく関与していることを報告してきた。そこでBLySがIgE産生クラススイッチに関与していないかどうか、そこに線維芽細胞の関与がないかどうかについて検討した。

アレルギー性鼻炎において抗原とIgEが肥満細胞上で架橋すると、細胞内シグナルが発生する。その代表的なシグナルとしてSykが有名である。我々は、Sykが血球系以外の細胞にも存在し、機能していること以前に見いだした。ヒト鼻粘膜由来線維芽細胞において、IL-1やTNFの刺激によってRANTESが産生されるが、その際Sykが重要なシグナルの一部であった。そこでアレルギー性鼻炎患者の鼻粘膜、鼻茸におけるSykの発現を検討した。

B. 方法

手術時に同時採取した鼻粘膜を培養し、線維芽細胞株を樹立した。Toll-like receptor(TLR), BLySの発現をreal-time PCRで測定し, 刺激後の発現の変化を検討した。刺激48時間後に培養上清を採取し、ウエスタンブロット法にてBLySの蛋白産生の変化を観察した。IgEクラススイッチに関しては、ヒトB細胞株Ramos2G6にGFP発現ベクターを遺伝子導入し、IL-4及び抗CD40抗体またはBLySで刺激後に, フローサイトメトリーにてGFP陽性細胞の数で定量比較した。ヒトB細胞をIL-4及びBLySで刺激し, 2日後のRNAを用い, RT-PCRでActivation-induced cytidine deaminase (AID)の発現を検討した。ヒトB細胞をIL-4及び抗CD40抗体で刺激し, 14日後の上清を採取し, IgEをELISAにて測定し, BlyS中和抗体前処理の場合と比較した。タンパク質リン酸化アレーとウエスタンブロットにより細胞内情報伝達を解明した。

同じように手術にて切除されたアレルギー性鼻炎患者と非アレルギー性鼻炎患者の鼻粘膜と鼻茸組織からホルマリン標本を作製し、免疫組織化学を行った。顕微鏡下でSykを発現している細胞をカウントし、比較検討した。また免疫二重染色を行い、Syk陽性細胞の同定を行った。

C. 結果

ヒトB細胞をIL-4及びBLySで刺激するとAIDの発現が誘導された。同様にIL-4と抗CD40抗体で刺激し, IgEをELISAにて測定すると, BLyS中和抗体前処理によりIgE産生が減少した。Igクラススイッチベクターを用い共刺激における実験では, 抗CD40抗体が低濃度ではBLySはIgEクラススイッチを有意に増強したが, 高濃度の存在下ではBLySの

影響はなかった。下甲介粘膜由来線維芽細胞ではTLR3, LTR4, TLR9の発現が多く, Poly ICとLPS刺激によりBLySが発現した。刺激48時間後に培養上清を採取し,ウエスタンブロット法にてBLySの蛋白産生も確認された。BLyS発現誘導は、Poly ICの作用が最も強く濃度依存性に誘導し、扁桃由来線維芽細胞でもみられたが、下甲介粘膜由来線維芽細胞で特に強くその１０倍であった。皮膚由来線維芽細胞ではBLyS発現みられず、組織特異性が認められた。Poly ICによよBLyS発現は、JPI3K阻害剤により抑制された。細胞内情報伝達をタンパク質リン酸化アレイで検討すると, Rho, Syk, Vav, c-Src, TRAF6, p-Selectinの関与が証明された。

アレルギー性鼻炎患者の鼻茸には、非アレルギー患者の鼻茸に比べ有意に多くのSyk陽性細胞が認められた。二重染色の結果、Syk陽性細胞は多くが好酸球であった。しかしアレルギー性鼻炎患者鼻粘膜の粘膜下層と鼻腺細胞においては、非アレルギー性鼻炎患者のそれとSyk陽性細胞数に違いはなかった。

D. 考察

ウイルス感染とアレルギー疾患の関係では、ＲＳウイルスやライノウイルスによる喘息の悪化などが報告されている。今回、鼻粘膜に非常に多く存在する線維芽細胞にウイルス感染を模倣したPoly IC刺激によって、BLySが発現され、BLyS自身弱いCD40刺激下では、IgEクラススイッチを促進する働きがあることが証明された。このことは、経験的に風邪などのウイルス感染が起こると、アレルギー症状は悪化し、鼻閉などが増強する現象と一致する。さらに弱いCD40刺激下とは、T細胞や肥満細胞が少ないもしくは弱い条件でも、鼻粘膜において線維芽細胞からのBLySによってIgEが産生される可能性が高くなることを示している。これまで線維芽細胞は好酸球遊走因子や好酸球を生存させるサイトカイン・ケモカイン産生が主として検討されていたが、、IgEクラススイッチにも関与している可能性が高いことは、大変興味深い。これまで抗BLyS抗体は、シェーグレン症候群・SLE・リウマチ性関節炎などの自己免疫疾患に臨床応用されている。今後BLySのアレルギー性鼻炎臨床病態との関連、影響について検討する。

アレルギー性鼻炎患者の鼻粘膜においては、上皮層でのSyk陽性である好酸球が重要な役割を担っている可能性が高い。粘膜下層での好酸球はあまり重要でない可能性がある。Sykに対する阻害薬が開発され、臨床治験も欧米で行われ、効果が報告された。その投与法として点鼻法がとられていたが、今回の鼻粘膜上皮でのSyk陽性細胞数の差はまさしく点鼻法の正しさを示しているのかもしれない。

E. 結論：鼻粘膜においては、BLySはIgEクラススイッチ促進因子として働いていた。BLyS中和抗体（抗BLyS抗体）がIgE産生を減少させたことから、アレルギー性鼻炎の治療薬としての期待が高まってきた。またBLySが関与するシグナルRho, Vav, c-Src, TRAF6, p-SelectinとSykを標的として分子治療もアレルギー性鼻炎の治療候補であると思われる。この後は、RNAiを利用した抑制系の開発や特定細胞を標的にした抗体との組み合わせによって効率的なシグナル抑制系を樹立すべきであると考えている。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況
1.　特許取得
なし
2.　実用新案登録
なし
3.　その他
なし

Collaborative Action of NF-κB and p38 MAPK Is Involved in CpG DNA-Induced IFN-α and Chemokine Production in Human Plasmacytoid Dendritic Cells[1]

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CpG DNA induces plasmacytoid dendritic cells (pDC) to produce type I IFN and chemokines. However, it has not been fully elucidated how the TLR9 signaling pathway is linked to these gene expressions. We examined the mechanisms involving the TLR9 and type I IFN signaling pathways, in relation to CpG DNA-induced IFN-α, IFN regulatory factor (IRF)-7, and chemokines CXCL10 and CCL3 in human pDC. In pDC, NF-κB subunits p65 and p50 were constitutively activated. pDC also constitutively expressed IRF-7 and CCL3, and the gene expressions seemed to be regulated by NF-κB. CpG DNA enhanced the NF-κB p65/p50 activity, which collaborated with p38 MAPK to up-regulate the expressions of IRF-7, CXCL10, and CCL3 in a manner independent of type I IFN signaling. We then examined the pathway through which IFN-α is expressed. Type I IFN induced the expression of IRF-7, but not of IFN-α, in a NF-κB-independent way. CpG DNA enabled the type I IFN-treated pDC to express IFN-α in the presence of NF-κB/p38 MAPK inhibitor, and chloroquine abrogated this effect. With CpG DNA, IRF-7, both constitutively and newly expressed, moved to the nuclei independently of NF-κB/p38 MAPK. These findings suggest that, in CpG DNA-stimulated human pDC, the induction of IRF-7, CXCL10, and CCL3 is mediated by the NF-κB/p38 MAPK pathway, and that IRF-7 is activated upstream of the activation of NF-κB/p38 MAPK in chloroquine-sensitive regulatory machinery, thereby leading to the expression of IFN-α. *The Journal of Immunology,* **2006, 177: 4841–4852.**

Dendritic cells (DC)[3] consist of a heterogeneous population of APC that regulates immune responses. They are characterized by surface markers and cytokines induced in response to inflammatory stimuli, including the ligands for TLRs (1), which recognize distinct families of pathogenic products. Among DC, plasmacytoid DCs (pDC) are a unique population exhibiting plasmacytoid morphology (2). The biological distinction of pDC is that they produce a large amount of IFN-α through the ligation of TLR9 (3) with bacterial DNA or its synthetic counterpart, the so-called oligoDNA containing unmethylated CpG motifs (CpG DNA) (4–6). They also produce chemokines (7–9); through the interplay with the IFN-α, pDC participate not only in innate immunity but also in adaptive immunity (2).

Due to the identification of pDC and the discovery of microbial inducers of IFN-α, IFN-α has recently been recognized as a multifunctional cytokine (2, 10). IFN-α, as a member of the type I IFN-family, deliver signals to the classical type I IFN pathway via IFN-αβ receptor, and consequently the genes whose promoters carry IFN-stimulated response elements (ISRE) are transcribed. IFN regulatory factor (IRF) 7 is one of the genes up-regulated by type I IFN (11–13). In pDC, however, IRF-7 is constitutively expressed (14–17). Its association with MyD88 and TNF receptor-associated factor 6 (TRAF6) has recently been reported to be a prerequisite for the activation of the IFN promoter (18, 19).

Following ligation of TLR9, the adaptor MyD88 recruits signaling mediators to activate NF-κB (20). Therefore, most of the chemokines whose gene expression requires the activation of NF-κB could be induced through the TLR9 signaling in pDC. However, to our knowledge, there has not yet been any report that referred to the integral participation of NF-κB in the expression of IFN-α and chemokines in CpG DNA-stimulated human pDC. Recently, it has been demonstrated that, besides ISRE, the human IRF-7 gene promoter has a NF-κB binding site (21), and the activation of NF-κB is required for EBV latent membrane protein 1- and TNF-α-induced IRF-7 expression (21, 22). Then, we speculated that, in the end-point of TLR9 signaling, the activation of NF-κB could be involved in the up-regulation of the gene expression of IRF-7, subsequently of IFN-α, and that of other NF-κB-dependent chemokines in human pDC.

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[3] Abbreviations used in this paper: DC, dendritic cell; pDC, plasmacytoid DC; ISRE, IFN-stimulated response element; IRF, IFN regulatory factor; TRAF6, TNF receptor-associated factor 6; ODN, oligonucleotide; Act D, actinomycin D; CHX, cycloheximide; PDTC, pyrrolidinedithiocarbamate; CAPE, caffeic acid phenethyl ester; DEX, dexamethasone; PMX, polymixin B; BDCA, blood DC Ag; SR, scavenger receptor.

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