

Original article

Expression of osteopontin in chronic rhinosinusitis with and without nasal polyps

Background: Osteopontin (OPN) is a multifunctional 34-kDa extracellular matrix protein that can influence the inflammatory process. However, the presence of OPN in human sinonasal mucosa and its roles in the inflammatory process of chronic rhinosinusitis (CRS) are not clear. This study investigated the expression of OPN in human sinonasal mucosa, its cytokine-driven expression regulation, and its effect on cytokine production in sinonasal mucosa.

Methods: Surgical samples were investigated by means of quantitative reverse transcriptase polymerase chain reaction for evaluation of OPN messenger RNA (mRNA) expression, and the presence and location of OPN protein expression were analyzed using immunohistochemistry. Furthermore, nasal explant culture was used to investigate the mutual regulatory interactions between interferon (IFN)- γ , interleukin (IL)-4, IL-5, IL-13, IL-1 β , and tumor necrosis factor (TNF)- α and OPN in sinonasal mucosa.

Results: Osteopontin expression was significantly upregulated in CRS tissues compared with control tissues. There was a further significant increase of OPN expression in patients with nasal polyps (NPs) and asthma. Immunohistochemistry revealed positive staining of OPN in epithelial cells, submucosal glands, infiltrating cells, and extracellular matrix. Osteopontin mRNA was induced by IFN- γ , IL-1 β , and TNF- α , but inhibited by IL-4 and IL-13. On the contrary, OPN induced IFN- γ , IL-4, IL-5, IL-13, IL-1 β , and TNF- α production in sinonasal mucosa.

Conclusions: The expression of OPN is upregulated in CRS. The mutual regulatory interactions between OPN and inflammatory cytokines suggest that OPN may play an important role in the pathogenesis of CRS.

**X. Lu*, X. H. Zhang*, H. Wang,
X. B. Long, X. J. You, Q. X. Gao,
Y. H. Cui, Z. Liu**

Department of Otolaryngology-Head and Neck Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China

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Zheng Liu
Department of Otolaryngology-Head and Neck Surgery
Tongji Hospital, Tongji Medical College
Huazhong University of Science and Technology
1095 Jiefang Avenue
Wuhan 430030
P.R. China

*Drs Lu and Zhang contributed equally to this article.

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Chronic rhinosinusitis (CRS) is one of the most frequently reported chronic diseases, which is covering a spectrum of diseases, including nasal polyps (NPs) (1). The complexity of CRS makes the CRS etiology study and CRS therapeutics very difficult. Several hypotheses concerning its pathogenesis have been proposed, such as chronic microorganism infection, inhalant or food allergy, sinus ostial blockage, and local immunological disturbance (2). Although these studies have increased our understanding of CRS, the exact etiology of CRS is still far from completely revealed. So far, one of the most important characteristics of CRS is the prolonged and exaggerated inflammatory reaction in paranasal mucous membrane. As a result of the effect on inflammatory cell recruitment and activation, and tissue remodeling; cytokine and chemokine are implicated in the development of CRS (2). A lot of studies have reported increased levels of interleukin (IL)-4, IL-5, IL-13, IL-1 β , and tumor necrosis factor (TNF)- α in the sinonasal mucosa from CRS patients with and without NPs (3, 4).

Recently, a novel cytokine, osteopontin (OPN), is catching scientists' eyes for its functions in recruiting

inflammatory cells into the local inflammatory site and regulating the function of these cells, such as monocyte/macrophage, dendritic cell, and T-cell (5–7). Osteopontin, also known as early T lymphocyte-activating gene-1 and secreted phosphoprotein I, is a phosphorylated acidic arginine-glycine-aspartate (RGD) containing glycoprotein that can bind certain CD44 variants and integrin receptors and mediate cell-matrix interactions and cellular signaling, involving both RGD-dependent and RGD-independent interactions (6). Expression of OPN is constitutive in bone and at epithelial surface and is upregulated in activated T-cells, macrophages, and tumor cells in model of inflammation, bone resorption, and tumor progression (5). Increasing evidence suggests that OPN may be tightly associated with inflammatory and immunological diseases. Osteopontin-deficient mice exhibit reduced immunity to viruses and other microorganisms (8). High levels of OPN have been observed in rheumatoid arthritis, Crohn's disease, and multiple sclerosis (9–11). However, at present, the role of OPN in the human airway under normal and pathologic conditions has received little attention. Recently, two studies have

shown that OPN might be involved in the allergic lower airway disease (12, 13). In the present study, we studied the possible role of OPN in CRS. Firstly, we examined the expression of OPN in sinonasal tissues from control and CRS patients by means of quantitative reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry. Furthermore, we used nasal explant culture and quantitative RT-PCR technique to assess the potential regulatory axis between OPN and CRS-relevant inflammatory cytokines in sinonasal mucosa.

Methods

Subjects

This study was approved by the ethical committee of Tongji Medical College of Huazhong University of Science and Technology and conducted with written informed consent from patients.

Seventy-two patients who underwent functional endoscopic sinus surgery or septal surgery were enrolled for quantitative RT-PCR and immunohistochemistry experiments. These patients were divided into three groups: controls, CRS patients without NPs, and CRS patients with NPs. Patients undergoing septoplasty because of anatomic variations and not having any sinus disease were considered control subjects and inferior turbinate mucosal samples were taken during surgery. Chronic rhinosinusitis with and without NPs was diagnosed according to the clinical criteria by Meltzer et al. on the basis of history, clinical examination, nasal endoscopy, and sinus-computed tomography scanning (14). Diseased sinus mucosal tissues and NP tissues were collected during surgery.

Additional inferior turbinate mucosal samples of 22 patients who underwent septal surgery and not having any sinus disease were obtained for nasal explant culture experiments. Clinical data of the patients are summarized in Table 1.

In this study, subjects were excluded if they had received any oral steroid or antihistamine 3 months before the surgery. Topical medications were withheld for a minimum of 1 month before study. None had received antileukotrienes and immunotherapy. Patients who underwent previous sinus surgery were excluded. Medical management strategy was identical in both CRS groups.

Table 1. Patients' clinical data

	Quantitative RT-PCR and immunohistochemistry			Nasal explant culture
	Control group	CRS without NPs	CRS with NPs	
No. of subjects	10	32	30	22
Sex (male/female)	6/4	19/13	16/14	14/8
Age (years)	18–46	18–59	16–62	17–55
No. of patients with asthma	0	5	8	0
No. of patients with positive skin prick test results	0	12	13	0
No. of patients with aspirin sensitivity	0	0	0	0

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

Human laryngeal squamous cell carcinoma tissues served as positive controls for quantitative RT-PCR and immunohistochemistry experiments.

Tissue preparation, immunohistochemistry, and quantification

Samples obtained during surgery were fixed in formalin and embedded in paraffin. Paraffin sections (4 µm) were prepared from each block and stained with Giemsa. Protein expression of OPN was examined by means of immunohistochemical staining using the streptavidin-peroxidase complex method under the manufacturer's instructions (15). Sections were stained with anti-OPN monoclonal antibody (1 : 100, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Color development was achieved with 3',3'-diaminobenzidine which rendered positive cells brown. Species- and subtype-matched antibodies were used as a negative control. To examine the relationship between OPN and eosinophil, the tissue sections positive for OPN staining were subjected to May-Grünwald Giemsa (MGG) staining after quantification and digital photographs were taken. The same visual field pre- and post-MGG staining was compared.

The number of total infiltrating cells, mononuclear cells, and eosinophils were determined by counting 10 randomly selected fields in a blinded fashion at 400 × magnification. Quantitative measurement of OPN protein expression was analyzed using the HPIAS-1000 automated image analysis system as described elsewhere (Olympus, Tokyo, Japan; 16). Ten microscopic fields were randomly selected from each slide under 400 × magnification. Results were presented as 1/gray scores. The gray score reflects the optical density of selected field for analyzing (16). So, 1/gray scores positively correlated with the intensity of immunoreactivity. The gray score of background was measured in nontissue area and subtracted from the gray score of each selected field.

Nasal explant culture

Normal inferior turbinate mucosal tissues were obtained during surgery and sectioned into multiple samples of approximately 6 mm³. One was processed for histologic evaluation and the others were used for tissue culture as described previously (16, 17). Briefly, sections of tissue were placed on 0.4 µm well inserts (Millipore Corp., Billerica, MA, USA) in 2 ml of tissue culture medium (18). The tissue was oriented with the epithelium being exposed to the air, forming an air-liquid interface to mimic the *in vivo* situation. Tissue was incubated in the absence or presence of various concentrations of interferon (IFN)-γ (0.1, 1, and 10 ng/ml), IL-4 (1, 10, and 100 ng/ml), IL-5 (1, 10, and 100 ng/ml), IL-13 (1, 10, and 100 ng/ml), IL-1β (1, 10, and 100 ng/ml), TNF-α (1, 10, and 100 ng/ml), or recombinant human osteopontin (rhOPN) (10 nM, 100 nM, and 1 µM) for various time durations between 4 and 24 h. All these cytokines were purchased from R&D Systems (Minneapolis, MN, USA). In some experiments, 5 µg/ml of actinomycin D (Sigma, St Louis, MO, USA) was added 12 h after stimulation with IFN-γ (10 ng/ml) or IL-4 (20 ng/ml). Total RNAs were isolated immediately (time = 0) or at 4, 8, 12, and 24 h after the addition of actinomycin D. The tissue was cultured at 37°C with 5% CO₂ in humidified air.

Quantitative reverse transcriptase polymerase chain reaction

Tissues were homogenized and RNA was extracted by using an RNeasy Mini kit (Qiagen, Valencia, CA, USA) and treated by using a DNA-free kit (Ambion, Austin, TX, USA) to remove contaminating DNA. cDNA was reverse transcribed from 0.5 µg of total

Table 2. Primer sequences used for quantitative RT-PCR analysis

Primer	Sequence	Annealing temperature (°C)
OPN	[s] 5'-CCAAGTAAGTCCAACGAAAG-3'	66
	[a] 5'-GGTGATGTCCTCGTCTGTA-3'	
GAPDH	[s] 5'-GAAGGTGAAGGTCGGAGTC-3'	66
	[a] 5'-GGAAGATGGTGTGGGATT-3'	
IFN- γ	[s] 5'-GCTTTTCAGCTCTGCATCGTT-3'	66
	[a] 5'-TTTCTTAAGGTTTCTGCTCTTTTA-3'	
IL-4	[s] 5'-AACAGCCTCAGAGCAGAAGAC-3'	66
	[a] 5'-GCCCTGCAGAAAGTTTCCCTT-3'	
IL-5	[s] 5'-TAGCTCTTGAGAGTCGCTACGT-3'	60
	[a] 5'-CAAGGTCTCTTCCACCAATGCA-3'	
IL-13	[s] 5'-AGGATGCTGAGCGGATTCTG-3'	60
	[a] 5'-AAACTGGGCCACCTCGATT-3'	
IL-1 β	[s] 5'-GATGAAAGACGGCACACC-3'	60
	[a] 5'-CTATGTCCTCCGACCATTCG-3'	
TNF- α	[s] 5'-TCTGGCCAGGCAGTCA-3'	60
	[a] 5'-GCTTGAGGGTTTGCTACAACATG-3'	

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; OPN, osteopontin; RT-PCR, reverse transcriptase polymerase chain reaction.

RNA with random hexamer primers. Quantitative PCR was performed on the LightCycler system (Roche Diagnostics, Mannheim, Germany) by using the SYBR Premix Ex Taq kit (TaKaRa Biotechnology, Dalian, China) with the appropriate primers (Table 2) and samples according to the manufacturer's protocol. In brief, 1 μ l cDNA was added to 10 μ l 2 $\times$ SYBR Premix Ex Taq master mix, 8 μ l RNase-free water, and 1 μ l of each primer (5 μ M), resulting in a total volume of 20 μ l. The PCR conditions consisted of an initial denaturation at 95°C for 30 s, followed by amplification for 45 cycles of 5 s at 95°C and at different annealing temperature for 20 s. After PCR, a melting curve was constructed by increasing the temperature from 65 to 95°C with a temperature transition rate of 0.1°C/s. Relative gene expression was calculated by using the comparative CT method (19). A sinus mucosa sample from one CRS patient without NPs was used as a calibrator in OPN messenger RNA (mRNA) expression study, whereas tissues without any treatment were employed as calibrators in the *ex vivo* culture study. Glyceraldehyde-3-phosphate dehydrogenase was used as a house-keeping gene for normalization and a 'no template' sample was used as a negative control.

Statistical analysis

Data are presented as mean $\pm$ SD. Paired sets of RT-PCR and immunohistochemistry data were compared with Kruskal–Wallis H and Mann–Whitney *U*-test. Paired *t*-test was used in tissue culture data analysis. Differences were considered statistically significant at a *P*-value of <0.05 .

Results

Osteopontin expression in chronic rhinosinusitis patients with and without nasal polyps

We found that mRNA and protein expression of OPN was significantly upregulated in tissues from CRS patients compared with control tissues ($P < 0.01$). Among CRS patients, patients with NPs showed significantly stronger mRNA and protein expression in sinonasal mucosa

than patients without NPs of OPN (Fig. 1A, B). When comparing asthmatic and nonasthmatic patients, we found a significant increase of OPN mRNA and protein expression in asthmatic patients in both CRS with and without NPs group. Moreover, asthmatic patients in CRS with NPs group had a significant higher expression of OPN than asthmatic patients in CRS without NPs group and a same trend was found when comparing nonasthmatic CRS patients with and without NPs (Fig. 1C, D). No difference in OPN levels was found between atopic and nonatopic patients, and between male and female patients (data not shown). Osteopontin positive staining was demonstrated in epithelial cells, extracellular matrix, submucosal glands, and infiltrating cells by means of immunohistochemical staining (Fig. 1E–J). Analyzing the relationship between OPN-staining intensity and the number of eosinophils, mononuclear cells, and total infiltrating cells in CRS patients with and without NPs, we only noticed a significant correlation between OPN expression and eosinophil cells in CRS patients with NPs ($r = 0.56$, $P < 0.01$). Employing OPN immunohistochemical staining and subsequent MGG staining, we found most eosinophils in lamina propria in NPs were OPN-positive cells (Fig. 1K, L).

The effect of cytokines on osteopontin messenger RNA production in sinonasal mucosa

To determine the potential factors contributing to the regulation of OPN gene expression in sinonasal mucosa, the modulation of OPN mRNA expression by selected Th1 (IFN- γ), Th2 (IL-4, IL-5, and IL-13), and proinflammatory cytokines (IL-1 β and TNF- α) was examined in normal inferior turbinate mucosal tissue using *ex vivo* culture. Osteopontin mRNA expression was examined by means of quantitative RT-PCR. Interferon- γ , IL-1 β , and TNF- α were found to enhance the levels of OPN mRNA expression in a time- and dose-dependent manner (Fig. 2A, B, G–J). The time course study showed the increase in OPN mRNA expression was detected 4 h after the addition of above cytokines, and the expression levels were almost close to the maximum around 8–12 h after stimulation. On the contrary, we found OPN mRNA expression levels were visibly inhibited by IL-4 and IL-13 in a dose-dependent manner. The decrease in OPN expression was detected 4 h after stimulation with IL-4 and IL-13 at 10 ng/ml and was nearly close to the maximum after 12 h stimulation in a time-dependent manner (Fig. 2C–F). No obvious effect of IL-5 on OPN mRNA expression was found (data not shown).

Interferon- γ and interleukin-4 effect on osteopontin messenger RNA stability

We chose one Th1 cytokine (IFN- γ) as well as one Th2 cytokine (IL-4), which significantly influence the OPN mRNA expression to examine whether the regulation of

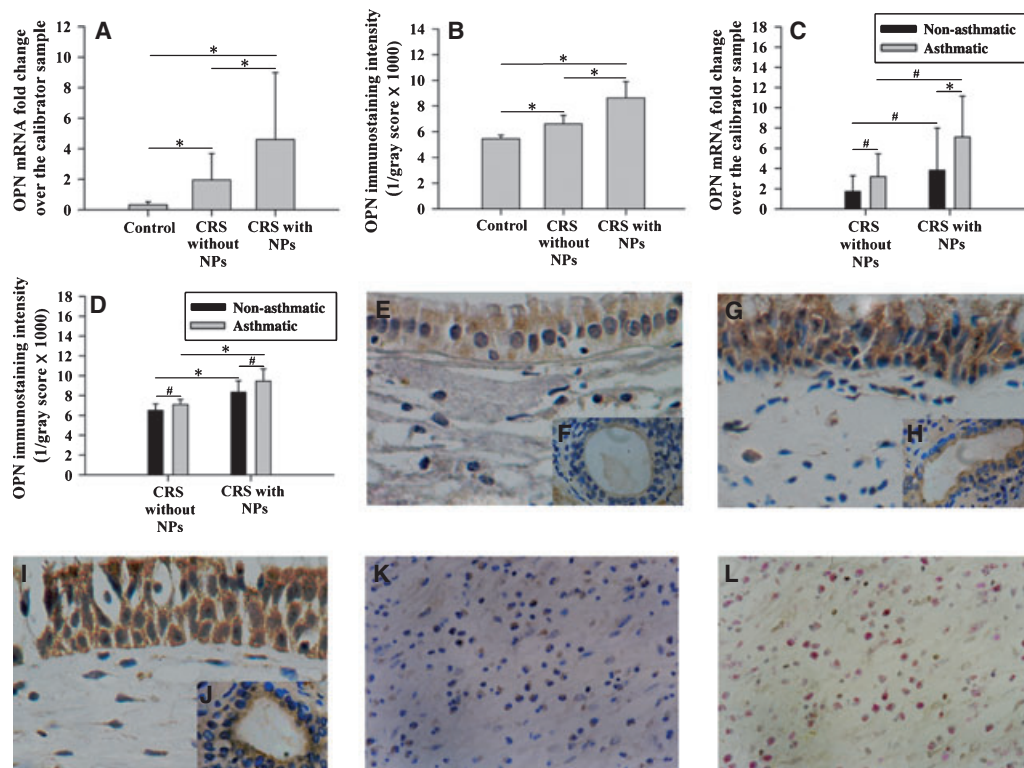


Figure 1. Expression of osteopontin (OPN) in sinonasal mucosa. (A–D) Quantification of OPN mRNA and protein expression in sinonasal mucosal tissues from controls and chronic rhinosinusitis (CRS) patients with and without nasal polyps (NPs) with quantitative reverse transcriptase polymerase chain reaction analysis (A and C) and immunohistochemistry (B and D), respectively; # $P < 0.05$ and * $P < 0.01$. (E–L) Representative photomicrographs of sinonasal tissue sections showing immunohistochemical staining. (E and F) OPN immunoreactivity in epithelial cells, extracellular matrix (E), and submucosal glands (F) of inferior turbinate mucosa from controls. (G and H) Obvious OPN immunoreactivity in epithelial cells, extracellular matrix (G), and submucosal glands (H) of sinonasal mucosa from CRS patients without NPs. (I and J) Obvious OPN immunoreactivity in epithelial cells, extracellular matrix (I), and submucosal glands (J) of NPs tissues. (K) OPN positive infiltrating cells in NPs tissues. (L) Same section as in (K) was restained with May–Grünwald Giemsa (MGG). Most OPN positive cells in (K) could be identified as eosinophils by MGG staining. (original magnification: $\times 400$).

OPN gene expression in sinonasal mucosa by these cytokines occurs at the post-transcriptional level. Normal nasal tissues were stimulated with IFN- γ (10 ng/ml) or IL-4 (20 ng/ml) for 12 h, when the greatest change of OPN mRNA expression was noted. This was followed by the addition of 5 μ g/ml actinomycin D. Total RNAs were isolated immediately (time = 0) or at 4, 8, 12, and 24 h after the addition of actinomycin D, and quantitative RT-PCR of OPN was performed. A decay curve was generated by plotting the ratios of normalized intensities relative to the respective samples at time = 0. The results showed that in tissues treated with or without selected cytokine, no significant alteration was seen in decay kinetics of the OPN transcripts after the addition of actinomycin D (Fig. 3).

The effect of osteopontin on cytokine messenger RNA production in nasal mucosa

To explore the role of OPN in upper airway inflammatory network, the production of IFN- γ , IL-4, IL-5, IL-13,

IL-1 β , and TNF- α induced by rhOPN was examined in normal inferior turbinate mucosa. We checked the concentration response and the time course of rhOPN on the mRNA expression of these selected cytokines, which was determined by means of quantitative RT-PCR. We found that all the cytokines mentioned above was induced by rhOPN in a time- and dose-dependent manner (Fig. 4). Among these cytokines, IFN- γ and IL-1 β showed more notable up-regulation with approximate 10-fold increase. (Fig. 4A, I).

Discussion

Although OPN has been implicated in various immunological events, its physiologic functions and potential roles in pathologic conditions remain to be defined. Currently, the evidence showing the relationship between OPN and human diseases is scarce. In the present study, we demonstrated for the first time that OPN expression was increased in CRS. Moreover, asthmatic patients had

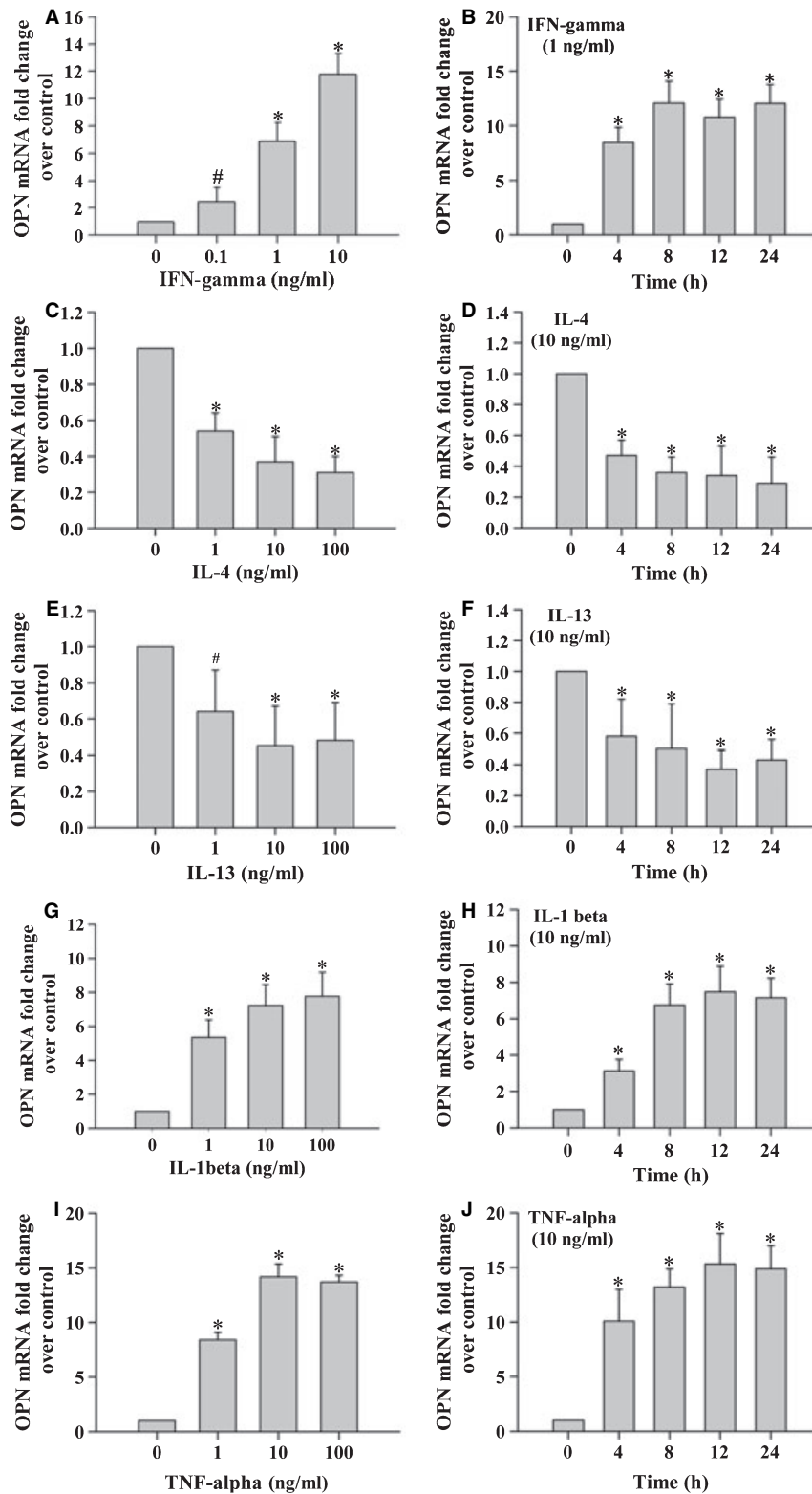


Figure 2. The effect of IFN- γ , IL-4, IL-13, IL-1 β , and TNF- α on osteopontin (OPN) mRNA expression in *ex vivo* cultured normal inferior turbinate mucosa. The tissues were incubated with selected cytokines at various concentrations for 12 h or at a certain concentration for various time durations. IFN- γ (A and B), IL-1 β (G and H), and TNF- α (I and J) enhanced, whereas IL-4 (C and D) and IL-13 (E and F) inhibited OPN mRNA expression in a time- and dose-dependent manner ($n = 6$). [#] $P < 0.05$ and ^{*} $P < 0.01$ compared with untreated mucosa.

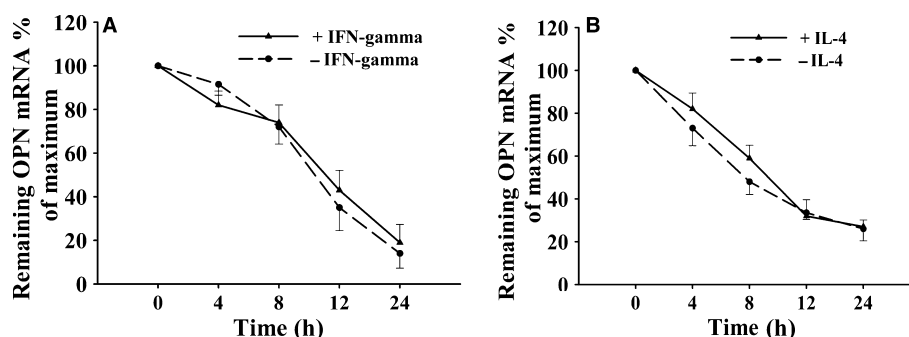


Figure 3. Decay kinetics of osteopontin (OPN) mRNAs in *ex vivo* cultured normal inferior turbinate mucosa. Normal inferior turbinate mucosa tissues were stimulated with IFN- γ (1 ng/ml) (A) or IL-4 (10 ng/ml) (B) for 12 h, followed by the addition of 5 μ g/ml actinomycin D. A decay curve was generated by plotting the ratios of normalized intensities relative to the samples at time = 0 ($n = 4$).

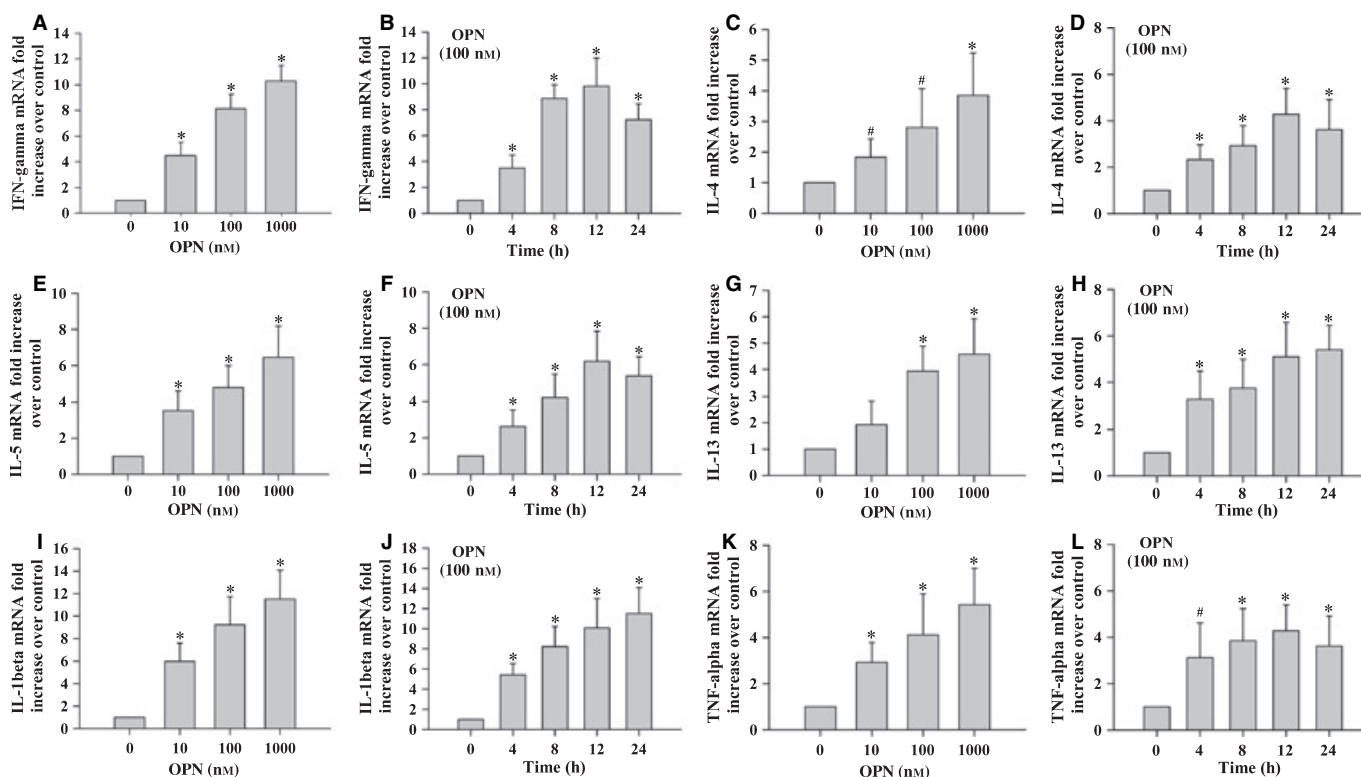


Figure 4. Treatment with recombinant human osteopontin (rhOPN) induced a significant upregulation in expression of IFN- γ (A and B), IL-4 (C and D), IL-5 (E and F), IL-13 (G and H), IL-1 β (I and J), and TNF- α (K and L) mRNA in *ex vivo* cultured normal inferior turbinate mucosa in a time- and dose-dependent manner ($n = 6$). The tissues were incubated with rhOPN at various concentrations for 12 h or at 100 nM for various time durations. # $P < 0.05$ and * $P < 0.01$ compared with untreated mucosa.

higher levels of OPN expression. As a corollary, enhanced OPN expression was found in lung biopsies from asthmatics in two recent studies (12, 13). We found that cells producing OPN in sinonasal mucosa included epithelial cells, submucosal glands cells, and infiltrating cells, which is in accordance with the finding in asthmatics (12, 13). Interestingly, we found that CRS patients with NPs had a significantly stronger OPN expression in sinonasal tissues

than CRS patients without NPs despite the coexistence of asthma or not, indicating OPN may have a higher impact on the pathogenesis of NPs. When we explored the correlations between OPN expression and different kinds of inflammatory cells in CRS with and without NPs, we could only find a significantly positive correlation between OPN expression intensity and the number of eosinophils in NPs. In NPs, there is a predominant

eosinophilic infiltration. To explore whether eosinophils are OPN-expressing cells, we conducted immunohistochemical staining and subsequent MGG staining and found that most eosinophils were OPN expressing cells in NPs, suggesting the eosinophil is an important source of OPN production in NPs.

Previous studies have demonstrated that the expression of OPN in various mice models and cell lines could be modulated by several cytokines such as Th1 (IFN- γ), Th2 (IL-4 and IL-13), and proinflammatory cytokines (TNF- α and IL-1 β) (5, 20, 21), but whether these cytokines are also involved in the OPN gene expression regulation in sinonasal mucosa is unclear. As these cytokines have been implicated in the pathogenesis of CRS (3, 4), we evaluated the regulation of OPN gene expression in sinonasal mucosa by these cytokines. Our immunohistochemical study revealed that OPN was not only expressed by epithelial cells, but also by submucosal glands and inflammatory cells in sinonasal mucosa; therefore, we generated sinonasal mucosa explants and did *ex vivo* culture, which simulated the *in vivo* nasal environment. We found the mRNA expression of OPN was significantly induced by IL-1 β , TNF- α , and IFN- γ , but inhibited by IL-4 and IL-13 in a dose- and time-dependent manner. In line with our results, OPN expression was shown to be inhibited by IL-4 and IL-13 in monocytes and dendritic cells and be enhanced by IFN- γ , IL-1 β , and TNF- α in macrophages (20, 21). As IL-1 β and TNF- α have been reported to be overexpressed in CRS (4), our results indicate that enhanced IL-1 β and TNF- α may contribute to the up-regulated expression of OPN in CRS. Recently, Van Zele and Bruaene compared the difference in inflammatory mediators expression between different chronic sinus diseases and discovered that CRS without NPs revealed a Th1 polarization with high levels of IFN- γ , whereas NPs presented a Th2 skewed eosinophilic inflammation with high IL-5, IL-13, and IgE concentrations (3, 22). Based on our present finding that OPN mRNA expression in sinonasal mucosa is induced by Th1 cytokines, but inhibited by Th2 cytokines, it is hard to explain why NPs with higher levels of Th2 cytokines show stronger OPN expression compared with CRS without NPs with higher levels of Th1 cytokines. However, it should be noted that although we discovered effect of these cytokines on OPN expression *in vitro*, their role in OPN gene regulation during the development of CRS *in vivo* still needs to be defined. On the contrary, besides the cytokines tested here, a lot of other inflammatory mediators involved in the pathogenesis of CRS may also contribute to the OPN gene regulation. Moreover, during the development of CRS *in vivo*, a lot of inflammatory cells will be recruited into sinonasal mucosa and may take part in OPN expression (22). Therefore, additional studies are required to investigate the mechanisms underlying the overexpressed OPN in CRS.

It is well established that mRNA stability plays a central role in the regulation of gene expression. To explore the mechanisms underlying the effect of cytokines on OPN expression, we examined the mRNA stability of OPN after stimulated with IFN- γ and IL-4. No significant alteration was seen in decay kinetics of the OPN transcripts after the addition of actinomycin D, suggesting that the regulation of OPN expression by IFN- γ and IL-4 is not mediated through the change of OPN mRNA stability. However, Konno et al. reported that faster decay of the OPN mRNA was seen in IL-4 treated monocytes (20). This discrepancy could result from the difference between monocytes and inferior turbinate tissue. Further studies on signal transduction and transcript factors of OPN gene will be helpful to elucidate the mechanisms underlying cytokine-driven expression of OPN.

Previous studies demonstrated that OPN is essential for IFN- α production by plasmacytoid dendritic cells (23). Osteopontin can induce IL-1 β and TNF- α production in human monocytes, and IL-4, IL-13, IL-12, and IL-10 levels were decreased in draining lymph nodes of allergic mice treated with OPN-specific antibody (12, 21). In the present study, we found that the mRNA expression of Th1, Th2, and proinflammatory cytokines could be induced by rhOPN in a time- and dose-dependent manner in sinonasal mucosa. It indicates that OPN may play a pivotal proinflammatory function in the inflammatory and immunological process in sinonasal mucosa. Previous studies have shown that OPN is a multifunctional molecule favoring Th1 immune response and has been considered as a Th1 cytokine by some scientists (5). Our present and recent studies from others indicate that OPN may have a wide range of functions and also be deeply involved in the Th2-derived diseases (12, 13).

Very interestingly, although we found OPN could induce Th2 cytokines mRNA expression in nasal mucosa, Th2 cytokines were shown to inhibit OPN mRNA expression, indicting a possible negative feedback loop may exist between OPN and Th2 cytokines in nasal airway inflammatory process.

Normal inferior turbinate tissue consists of epithelium, submucosal glands, infiltrating cells, fibroblasts, and extracellular matrix. Although *ex vivo* culture experiment can mimic the *in vivo* nasal environment, it is hard to tell which component exerts major effect using this study method. Further study employing specific cell lines is needed to deepen our understanding of OPN's function in CRS.

Conclusions

The expression of OPN is upregulated in CRS with and without NPs. There was a significant further increase of OPN expression in patients with NPs and asthma. The mutual regulatory interactions between OPN and inflammatory cytokines in sinonasal mucosa suggest that OPN

may play an important role in the pathogenesis of upper airway diseases. However, further studies are warranted to reveal the exact role of OPN in CRS with and without NPs.

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Conflict of Interest

None

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