

Letter to the Editor

Association of periostin expression with eosinophilic inflammation in nasal polyps

To the Editor:

Periostin has received much attention recently as a marker of T_H2 -biased eosinophilic disease and has been suggested to play a regulatory role in eosinophilic inflammation in several studies.¹ T_H2 cytokines and eosinophilic infiltration have generally been reported to be more specific for chronic rhinosinusitis with nasal polyps (CRSwNP) than for chronic rhinosinusitis without nasal polyps (CRSSNP).² Although periostin expression has been shown to be increased in patients with CRSwNP,³ its role in CRSwNP has not yet been fully elucidated.

The aim of this study was to investigate the involvement of periostin in eosinophil infiltration and localization in nasal polyps

(NPs) of patients with CRSwNP. Details of the materials and methods used in this study are shown in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Our study confirmed that tissue periostin expression was upregulated in patients with CRSwNP (Fig 1, A-C) and that periostin expression was not influenced by atopic status (see Fig E1 in this article's Online Repository at www.jacionline.org) or comorbid asthma in a subject (Fig 1, A). Our study further demonstrated that tissue periostin expression was significantly higher in IL-5^{high} patients with CRSwNP than in IL-5^{low} patients with CRSwNP, patients with CRSSNP, or control subjects (Fig 1, A-C). Also, tissue periostin concentrations were significantly correlated with IL-5 expression in patients with CRSwNP ($r = 0.406$, $P = .013$), thus supporting the view that periostin expression might be associated with a specific CRSwNP subtype with high mucosal IL-5 expression. However, there was no

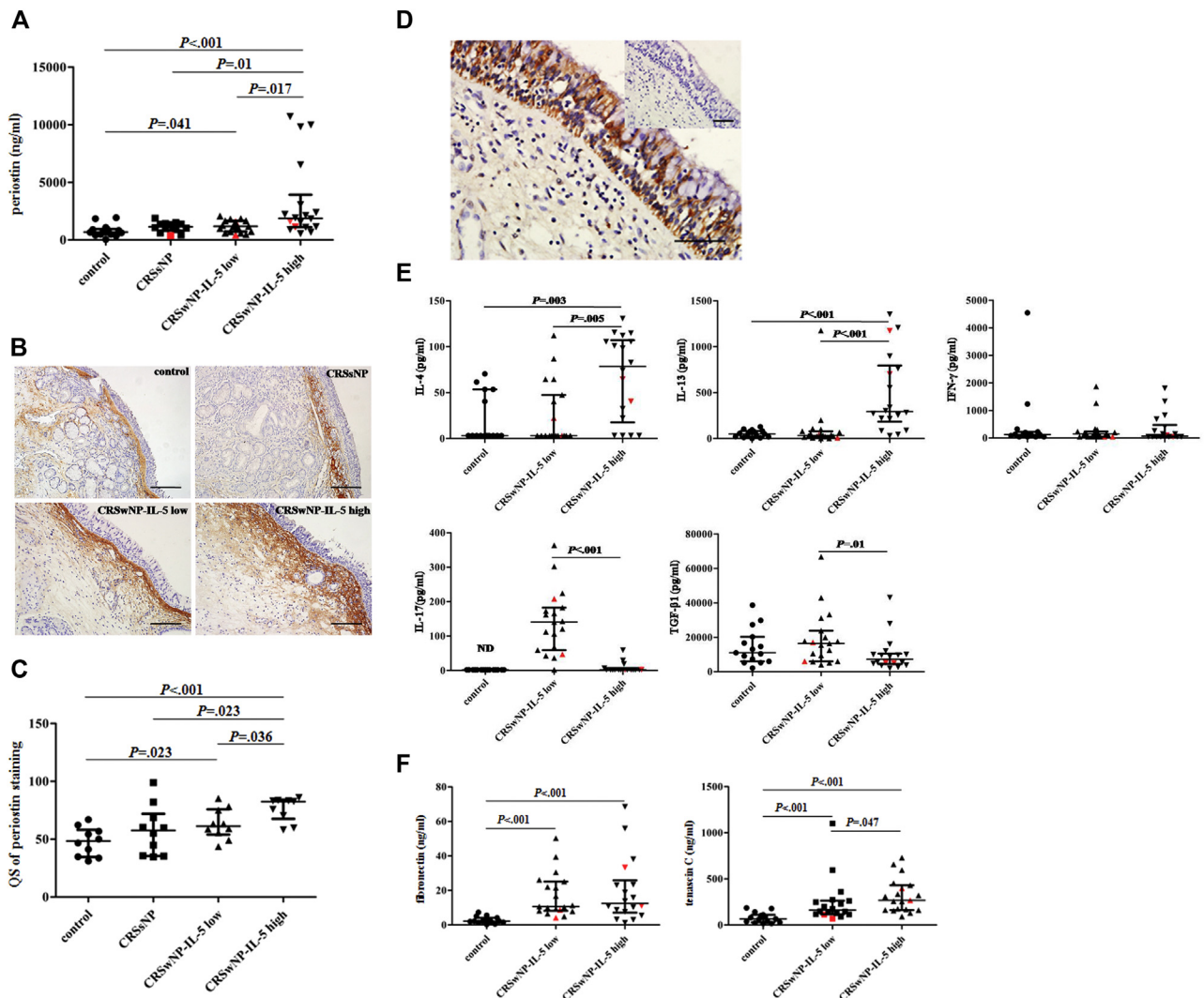


FIG 1. A, Periostin concentrations in tissue homogenates. B and C, Periostin staining in nasal tissue (Fig 1, B) and quantitative analysis (Fig 1, C). Bars = 40 μ m. D, In situ hybridization for periostin mRNA in NP tissue. Inset, Sense probe. Bar = 20 μ m. E and F, Concentrations of cytokines (Fig 1, E) and ECM proteins (Fig 1, F) in tissue homogenates. Patients with comorbid asthma are indicated by red points. ND, Not detectable.

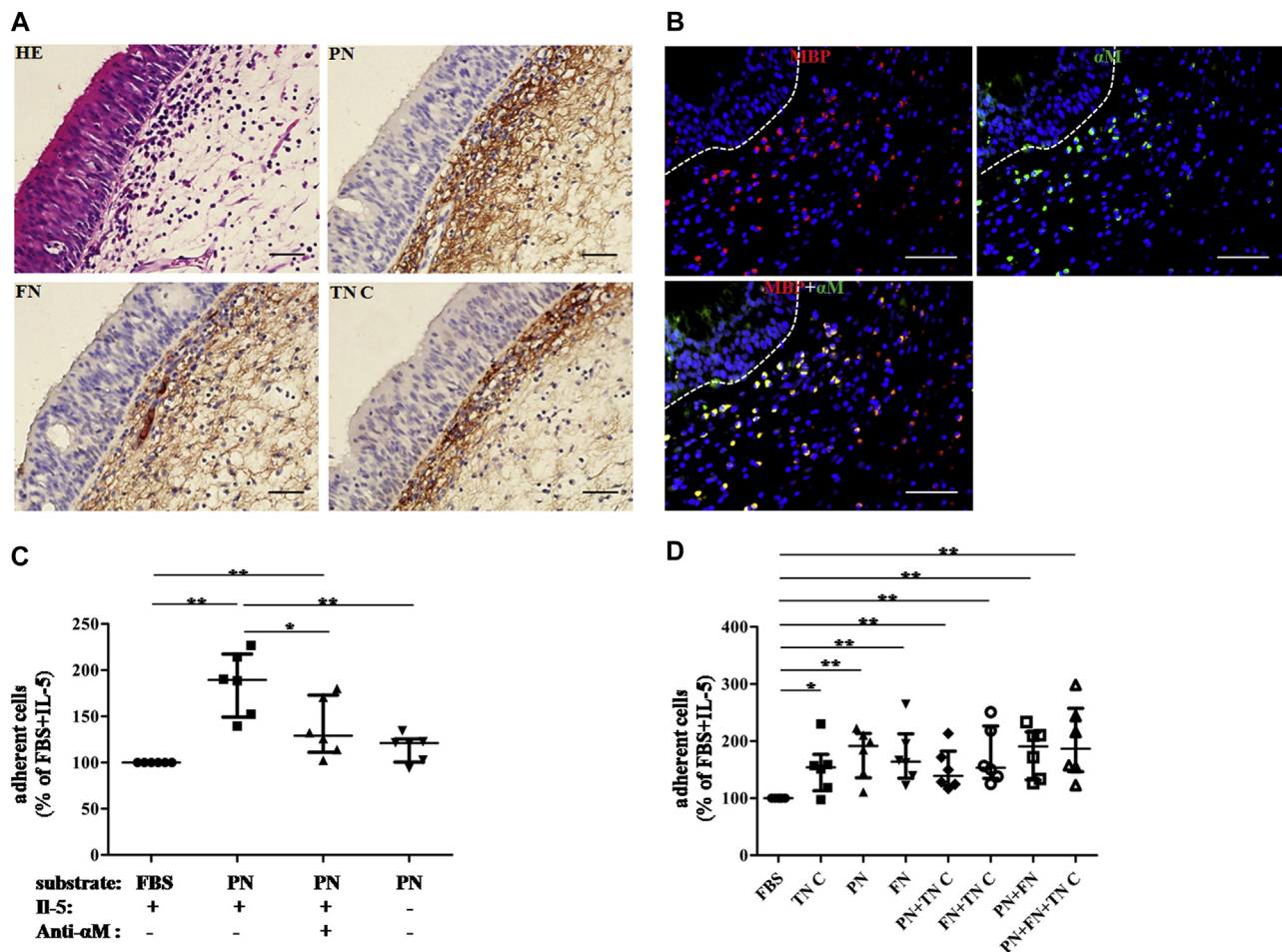


FIG 2. **A**, Hematoxylin and eosin (HE) and ECM protein staining in NP tissues. Bars = 40 μ m. **B**, Major basic protein and α M staining in NP tissues. Bars = 20 μ m. White dotted line indicates the basement membrane. **C** and **D**, Eosinophil adhesion to periostin (PN; Fig 2, C) or other ECM proteins (Fig 2, D). * $P < .05$ and ** $P < .01$ ($n = 6$). FN, Fibronectin; TN C, tenascin C.

difference for serum periostin concentrations between IL-5^{low} and IL-5^{high} patients with CRSwNP (see Fig E2 in this article's Online Repository at www.jacionline.org), suggesting that serum periostin expression does not suitably reflect local mucosal expression.

Immunostaining showed that periostin protein was mainly expressed in the subepithelial regions of patients with CRSwNP but not in the epithelium (Fig 1, B). In contrast, *in situ* hybridization showed periostin transcripts to be mainly expressed in the epithelium of NP tissue (Fig 1, D), which is consistent with a previous study in asthmatic patients⁴ and suggests that periostin expression in patients with CRSwNP is produced mainly by epithelial cells and deposited into the subepithelial region.

Periostin expression has been shown to be regulated by several mediators, including TGF- β 1, TGF- β 2, and TGF- β 4; bone morphogenic proteins 2 and 4; vascular endothelial growth factor; and IL-3, IL-4, IL-6, and IL-13.⁵ Our study showed that concomitant with the significantly increased periostin expression in nasal tissue of IL-5^{high} patients with CRSwNP, expression of IL-4 and IL-13, but not IFN- γ , IL-17, and TGF- β 1 (Fig 1, E), was also significantly increased in IL-5^{high} patients with CRSwNP compared with that seen in IL-5^{low} patients with

CRSwNP and control subjects, with a significant correlation between periostin and IL-4 expression ($r = 0.342$, $P = .038$). Assessment of IL-4-, IL-13-, and IL-5-induced periostin expression in tissue from patients with CRSwNP *ex vivo* further demonstrated that IL-4 and IL-13, but not IL-5, induced periostin expression in polyp tissue (see Fig E3 in this article's Online Repository at www.jacionline.org). These findings are in accordance with the findings of another study that demonstrated IL-4- and IL-13-induced upregulation of periostin expression in bronchial epithelial cells and fibroblasts of asthmatic patients.⁶

Next, we investigated the association of periostin expression with well-documented regulators and markers of eosinophil activity. First, our study confirmed that tissue IL-5 and eotaxin expression both correlated with tissue eosinophil cationic protein (ECP) levels and tissue eosinophil numbers in patients with CRSwNP, with tissue IL-5 expression also being correlated with blood eosinophil numbers (see Table E1 in this article's Online Repository at www.jacionline.org). In contrast, tissue periostin expression was correlated with IL-5 expression, but not with eotaxin (data not shown) or tissue ECP expression or tissue or blood eosinophil numbers (see Table E1), suggesting that periostin was unlikely to play a major role in the recruitment of

eosinophils into NPs. These findings are in contrast to the findings in asthmatic patients¹ but consistent with a study in animals, which showed no decrease in eosinophilic infiltration to nasal tissue in periostin-null mice challenged with ovalbumin and additional *Staphylococcus aureus* enterotoxin B.⁷

However, colocalization experiments indicated the presence of both eosinophils and periostin in the subepithelial region of NP tissue (Fig 2, A), with most major basic protein-positive eosinophils close to the epithelial barrier expressing the integrin α M subunit (Fig 2, B). This is in keeping with the collective findings from a previous study, which showed IL-5 to upregulate expression of eosinophil α M,⁸ and the present study, which shows that IL-5 expression is correlated with periostin expression. Furthermore, we demonstrated that periostin adhered to IL-5-stimulated eosinophils purified from patients with CRSwNP and that treatment with anti- α M antibody significantly attenuated adhesion of IL-5-stimulated eosinophils to periostin (Fig 2, C). These findings are also in accordance with the findings of Johansson et al,⁹ who demonstrated inhibition of IL-5-stimulated purified human blood eosinophil adhesion to periostin by mAbs to specifically α M or β_2 -integrin subunits. Collectively, these data provide evidence that periostin expression might be associated with eosinophil location in NPs and that this process is reliant on increased expression of α M β_2 on eosinophils.

Periostin has been reported to interact with extracellular matrix (ECM) proteins, such as type I collagen, fibronectin, tenascin C, and heparin.¹⁰ Accordingly, our results showed that the expression patterns of fibronectin and tenascin C were similar to that of periostin (Fig 1, F), with tenascin C expression correlated with periostin expression ($r = 0.397$, $P = .015$) in patients with CRSwNP and fibronectin and tenascin C expression colocalized with eosinophil counts and periostin expression in the subepithelial region of NP tissue (Fig 2, A). Moreover, eosinophils adhered to fibronectin and tenascin C (Fig 2, D). Although soluble periostin has been shown to increase adhesion of eosinophils to adsorbed fibronectin,⁹ no additive effects were observed in the present study by using various combinations of periostin, fibronectin, and tenascin C (Fig 2, D). Thus these data lend support to the hypothesis that periostin might interact with tenascin C and fibronectin to form the ECM reticular structure in patients with CRSwNP and that this interaction provides support to eosinophil migration without regulating eosinophil adhesion to ECMs. The upregulated expression of tenascin C mRNA in the IL-13-stimulated whole NP tissues from patients with CRSwNP (see Fig E4 in this article's Online Repository at www.jacionline.org) suggests that IL-13 might induce the expression of tenascin C in patients with CRSwNP.

In conclusion, this study indicates that T_H2 cytokine-mediated periostin expression is likely to be associated with eosinophil localization rather than eosinophil recruitment in tissue from patients with CRSwNP and that integrin expression on eosinophils and overexpression of ECM proteins are likely to be involved in this process. These findings might be helpful in

developing novel therapeutic methods for inhibiting eosinophilic inflammation in patients with CRSwNP.

Min Wang, PhD^{a,b,*}

Xiangdong Wang, MD, PhD^{a,b,c,*}

Nan Zhang, MD, PhD^d

Hong Wang, BS^b

Ying Li, BS^b

Erzhong Fan, BS^b

Liang Zhang, MS^b

Luo Zhang, MD, PhD^{a,b,c}

Claus Bachert, MD, PhD^d

From the Departments of ^aOtolaryngology–Head and Neck Surgery and ^cAllergy, Beijing TongRen Hospital, Capital Medical University, Beijing, China; ^bthe Beijing Key Laboratory of Nasal Diseases, Beijing Institute of Otolaryngology, Beijing, China; and ^dthe Upper Airways Research Laboratory, Department of Oto-Rhino-Laryngology, Ghent University Hospital, Ghent, Belgium. E-mail: dr.luozhang@139.com.

*These authors contributed equally to this study.

Supported by grants from the Program for Changjiang Scholars and Innovative Research Team (IRT13082), the National Natural Science Fund for the major international joint research program (81420108009), the Beijing Natural Science Foundation (7131006), the Ministry of Health Foundation (201202005), the 12th Five-Year Science and Technology Support Project (2014BAI07B04), the Capital Health Research and Development Program (2011-1017-06), and the Beijing Health Bureau Program for High Level Talents (2009-2-007, 2011-3-043 and 2011-3-039; to L.Z. and X.W.).

M. Wang, X. Wang, and L. Zhang received research support from the Program for Changjiang Scholars and Innovative Research Team, the National Natural Science Fund for The National Natural Science Fund for the Major International Joint research program, the Beijing Natural Science Foundation, the Ministry of Health Foundation, the 12th Five-Year Science and Technology Support Project, the Capital Health and Research and Development Program, and the Beijing Health Bureau for High Level Talents. The rest of the authors declare that they have no relevant conflicts to interest.

REFERENCES

- Li W, Gao P, Zhi Y, Xu W, Wu Y, Yin J, et al. Periostin: its role in asthma and its potential as a diagnostic or therapeutic target. *Respir Res* 2015;16:57.
- Van Zele T, Claeys S, Gevaert P, Van Maele G, Holtappels G, Van Cauwenberge P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy* 2006;61:1280-9.
- Zhang W, Hubin G, Endam LM, Al-Mot S, Filali-Mouhim A, Desrosiers M. Expression of the extracellular matrix gene periostin is increased in chronic rhinosinusitis and decreases following successful endoscopic sinus surgery. *Int Forum Allergy Rhinol* 2012;2:471-6.
- Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, et al. Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* 2010;107:14170-5.
- Norris RA, Moreno-Rodriguez R, Hoffman S, Markwald RR. The many facets of the matricellular protein periostin during cardiac development, remodeling, and pathophysiology. *J Cell Commun Signal* 2009;3:275-86.
- Takayama G, Arima K, Kanaji T, Toda S, Tanaka H, Shoji S, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 2006;118:98-104.
- Kim SW, Kim JH, Jung MH, Hur DG, Lee HK, Jeon SY, et al. Periostin may play a protective role in the development of eosinophilic chronic rhinosinusitis with nasal polyps in a mouse model. *Laryngoscope* 2013;123:1075-81.
- Barthel SR, Johansson MW, McNamee DM, Mosher DF. Roles of integrin activation in eosinophil function and the eosinophilic inflammation of asthma. *J Leukoc Biol* 2008;83:1-12.
- Johansson MW, Annis DS, Mosher DF. α M β 2 integrin-mediated adhesion and motility of IL-5-stimulated eosinophils on periostin. *Am J Respir Cell Mol Biol* 2013;48:503-10.
- Kudo A. Periostin in fibrillogenesis for tissue regeneration: periostin actions inside and outside the cell. *Cell Mol Life Sci* 2011;68:3201-7.

<http://dx.doi.org/10.1016/j.jaci.2015.09.005>

METHODS

Patients

Sixty-six subjects, including 14 patients with CRSsNP, 37 patients with CRSwNP, and 15 control subjects (Table E2), were recruited from the Rhinology Department of Beijing TongRen Hospital from 2011 to 2012. The study was approved by the Ethics Committee of Beijing TongRen Hospital, and written informed consent was obtained from all subjects before enrollment in the study.

Diagnosis of CRSsNP and CRSwNP was based on history, clinical examination, and results of nasal endoscopy and computed tomographic scanning according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012 guidelines for chronic rhinosinusitis.^{E1} Subjects who had taken systemic or local glucocorticosteroids or antibiotics within the last 4 weeks were excluded from the study. Subjects undergoing septoplasty or rhinoseptoplasty because of anatomic variations were recruited as control subjects. Atopic status was evaluated by using skin prick tests to various common inhalant allergens, and patients were given a diagnosis of asthma by a chest physician.

Tissue samples were obtained from the inferior turbinates of control subjects, the ethmoid mucosae of patients with CRSsNP, and the NPs of patients with CRSwNP, respectively. Nasal tissues were immediately frozen in liquid nitrogen and stored at -70°C until use for immunoassays. For immunostaining, tissue samples were fixed in 10% neutral buffered formalin for 24 hours and then embedded in paraffin.

Immunoassay

Tissue homogenates were prepared, as previously described.^{E2} Frozen tissues were weighed and homogenized with an automated homogenizer (TissueLyser LT; Qiagen, Dublin, Ireland) for 2 minutes in 0.9% NaCl (1 mL of 0.9% NaCl per 0.1 g of tissue) supplemented with 1% protease inhibitor cocktail (Sigma-Aldrich, St Louis, Mo). After homogenization, samples were centrifuged at 3000 rpm for 10 minutes, and supernatants were collected and stored at -70°C until assayed.

Periostin, ECP, eotaxin, IL-4, IL-5, IL-13, TGF- β 1, IL-17, IFN- γ , fibronectin, and tenascin C expression in tissue homogenates and periostin expression in serum were measured with commercially available kits, as detailed in Table E3.

Histologic analysis of nasal tissue

Nasal mucosal samples from control subjects and patients with chronic rhinosinusitis were assessed by means of immunohistochemical staining for periostin (Abcam, Cambridge, Mass). Results were quantitatively analyzed with image analysis software (IPP6; Media Cybernetics, Silver Springs, Md). Parameters measured were mean optical density (MOD; ie, the MODs of positive tissue areas) and staining index (SI; ie, the percentage of positive tissue area). The quick score (QS; $\text{QS} = \text{MOD} \times \text{SI}/100$) was used to represent the concentration of the stain. Five fields in each section randomly obtained at $\times 40$ magnification were evaluated.

Serial sections of NP samples from patients with CRSwNP were assessed by means of HE staining for eosinophils and by means of immunohistochemical staining for periostin, fibronectin (Dako, Glostrup, Denmark), and tenascin C (Abcam). Horseradish peroxidase-conjugated secondary antibodies (Zhongshanjinqiao, Beijing, China) and 3, 3'-diaminobenzidine used as substrate were used to visualize periostin, fibronectin, and tenascin C in the sections as brown-stained cells.

NP samples from patients with CRSwNP were also assessed by means of immunofluorescence double staining for major basic protein (Millipore, Bedford, Mass) and the integrin α M subunit (R&D Systems, Minneapolis, Minn). Positive staining was visualized with fluorescein isothiocyanate- and rhodamine-conjugated secondary antibodies, which rendered the cells green and red, respectively (Zhongshanjinqiao). Cell nuclei were stained with 4'-6-diamidino-2-phenylindole dihydrochloride.

Tissue eosino-phils were visualized by means of HE staining and counted in 10 different high-power fields ($\times 400$ magnification).

In situ hybridization

In situ hybridization was carried out in formalin-fixed paraffin-embedded tissue sections from NP biopsy specimens of patients with CRSwNP. Digoxigenin-labeled antisense and sense riboprobes were generated from a 364-bp cDNA fragment subcloned from full-length human periostin cDNA (position 198-562, accession no. NM006475.2).^{E3} The sections were incubated with a horseradish peroxidase-conjugated anti-digoxigenin antibody and stained with 3, 3'-diaminobenzidine.

IL-4-, IL-5-, and IL-13-induced expression of periostin, fibronectin, and tenascin C in NP tissue in vitro

Freshly obtained NP tissues from patients with CRSwNP were cut into approximately 1-mm³ fragments in RPMI 1640 culture media. The fragments were resuspended in fresh medium at 0.2 g/mL culture medium, and 0.5 mL of the suspension was placed into separate wells of a 24-well tissue-culture plate. The tissue fragments were incubated with 50 ng/mL IL-4, IL-5, or IL-13 (all from R&D Systems) for 24 hours, and then tissue fragments were collected and periostin, fibronectin, and tenascin C expression in tissue homogenates was measured by means of ELISA and real-time PCR.

Real-time PCR

Total RNA from tissue fragments was extracted with TRIzol reagent (Ambion-Life Technologies, Carlsbad, Calif). The quality of total RNA was assessed by using RNA electrophoresis. RNA was reverse transcribed into first-strand cDNA with oligo (dT) primer, and real-time PCR was performed with the ABI7500 PCR system (Applied Biosystems, Foster City, Calif). Expression of the periostin gene was determined by using the Platinum SYBR Green qPCR SuperMix UDG (Invitrogen, Carlsbad, Calif). Primer sequences were as follows: forward primer CGGGCAAATACTGGAAACCATC and reverse primer ACCGTTTCTCCCTTGCTTACTCC for periostin; forward primer AGACCATACCCGCGAATGTAGGAC and reverse primer CCTCTGCTGGTCTTTCAGTGCCTCC for fibronectin; forward primer GGCATTGGCTATGAGGTTATGGTCT and reverse primer TTCGGGG GCAAGTAGGGTTATTT for tenascin C; forward primer ACTTAGTT GCGTTACACCTT and reverse primer GTCACCTTCACCGTTCCA for the housekeeping gene β -actin. The comparative cycle threshold ($\Delta\Delta\text{Ct}$) method was used for relative gene expression analysis.

Isolation of eosinophils and cell adhesion

Eosinophils were purified from blood of patients with CRSwNP by using Percoll density gradient centrifugation and an eosinophil isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the standard protocol.

Ninety-six-well plates were coated with 100 μL of the relevant protein (5 $\mu\text{g}/\text{mL}$) or protein combinations in PBS for 2 hours at 37°C , decanted, and blocked with 100 μL of neat FBS. Control wells were coated with only FBS. Eosinophils (100 μL of 2×10^5 cells/mL) were added in the presence of recombinant IL-5 (10 ng/mL). In certain groups eosinophils were added in the absence of IL-5 or preincubated with 10 $\mu\text{g}/\text{mL}$ anti- α M (Abcam) for 5 minutes before adding to the wells. After 1 hour, wells were washed 3 times with PBS to remove unbound cells. Adherent cells were labeled with Calcein-AM fluorescent dye (Sigma) and quantified with a fluorescence plate reader (Mithras LB940; Berthold Technologies GmbH, Bad Wildbad, Germany) at excitation and emission wavelengths of 485 and 535 nm/L, respectively. Adherent cells were expressed as the percentage of cells adhering to a coating of FBS alone in the presence of IL-5 (10 ng/mL).

Statistical analysis

One-way ANOVA or the Fisher exact test was used to analyze baseline variables. Multiple comparisons among the different groups were analyzed by using the Kruskal-Wallis test and the nonparametric Mann-Whitney *U* test. Relationships between the various parameters were evaluated by using Spearman correlation analysis. The Wilcoxon matched pairs test was used to compare the expression values of patient-matched unstimulated and stimulated NP tissue. SPSS software for Windows (SPSS, Chicago, Ill) was applied for data analysis. A *P* value of less than .05 was considered statistically significant.

REFERENCES

- E1. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology* 2012;50:1-12.
- E2. Van Crombruggen K, Holtappels G, De Ruyck N, Derycke L, Tomassen P, Bachert C. RAGE processing in chronic airway conditions: involvement of *Staphylococcus aureus* and ECP. *J Allergy Clin Immunol* 2012;129:1515-21.
- E3. Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, et al. Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* 2010;107:14170-5.

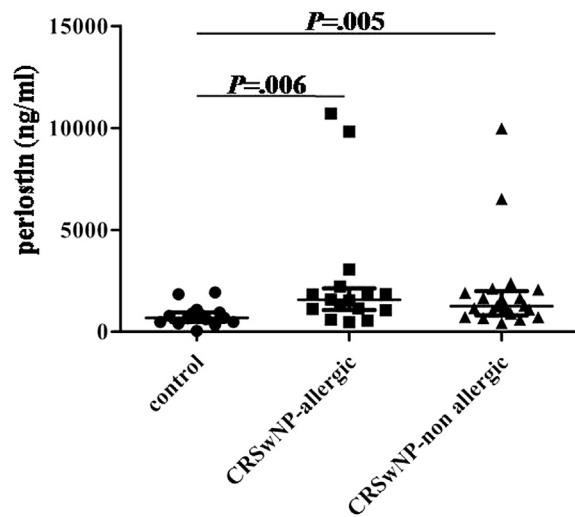


FIG E1. Concentrations of periostin in nasal tissue homogenates of patients with CRSwNP and control subjects. Patients with CRSwNP were divided into allergic patients with CRSwNP and nonallergic patients with CRSwNP based on skin prick test results. Control subjects, $n = 15$; allergic patients with CRSwNP, $n = 16$; nonallergic patients with CRSwNP, $n = 21$.

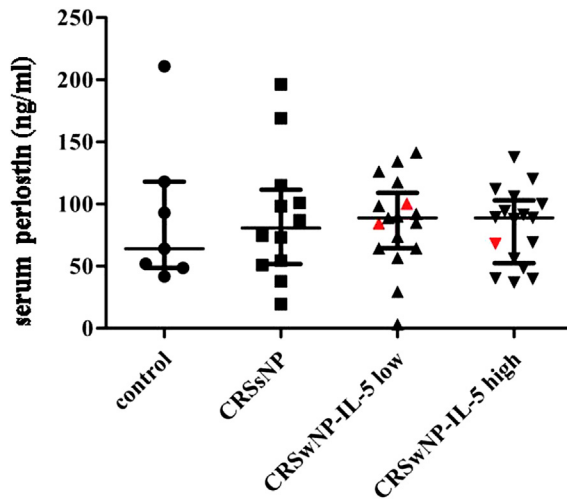


FIG E2. Serum periostin concentrations in patients with chronic rhinosinusitis and control subjects. Patients with comorbid asthma are indicated by *red points*. Control subjects, $n = 7$; patients with CRSsNP, $n = 12$; IL-5^{low} patients with CRSwNP, $n = 17$; IL-5^{high} patients with CRSwNP, $n = 17$.

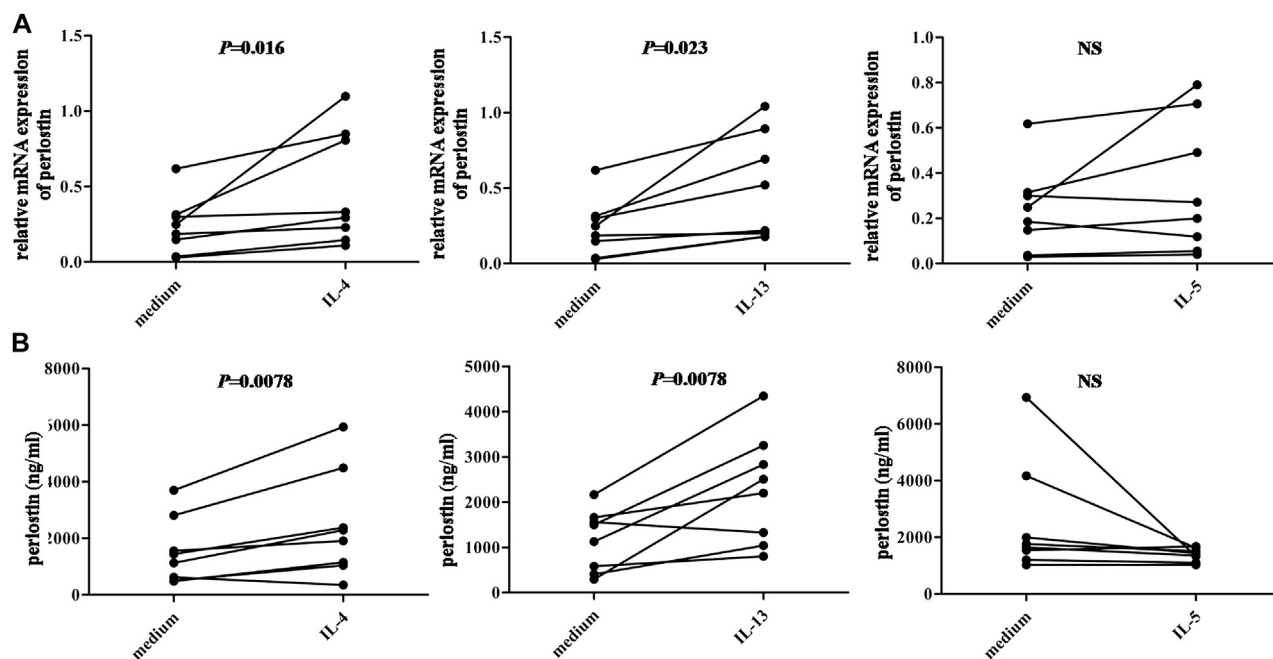


FIG E3. Periostin mRNA (**A**) and protein (**B**) expression in tissue homogenates of NP fragments incubated with IL-4, IL-5, or IL-13 ($n = 8$). Significance of differences in expression was assessed by using the Wilcoxon matched pairs test. *NS*, Not significant.

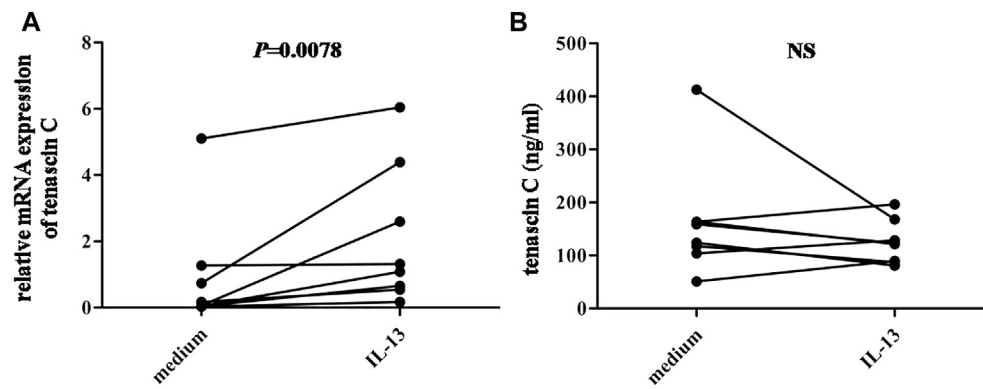


FIG E4. Induction of tenascin C in whole NP tissues by IL-13. Tenascin C mRNA (A) and protein (B) expression in tissue homogenates of NP fragments after IL-13 stimulation is shown (n = 8). Significance of differences in expression was assessed by using the Wilcoxon matched pairs test. *NS*, Not significant.

TABLE E1. Correlation of periostin, IL-5, and eotaxin expression in tissue homogenates with eosinophilic markers in patients with CRSwNP

Variable	Tissue ECP (<i>r</i> value/ <i>P</i> value)	Tissue eosinophil numbers (<i>r</i> value/ <i>P</i> value)	Blood eosinophil numbers (<i>r</i> value/ <i>P</i> value)
Tissue periostin	NS	NS	NS
Tissue IL-5	0.711/.000	0.422/.013	0.608/.000
Tissue eotaxin	0.478/.003	0.411/.016	NS

NS, Not significant.

TABLE E2. Characteristics of participants

Characteristic	Control subjects	Patients with CRSsNP	Patients with CRSwNP	ANOVA (Fisher exact test*)
No.	15	14	37	
Age (y)	42 (18-61)	47 (13-61)	46 (15-67)	NS
Female/male sex	7/8	11/3	28/9	NS*
Asthma	0/15	1/14	4/37	NS*
Positive skin prick test response	0/15	4/14	15/37	.042*† .002‡
Aspirin intolerance	0/15	0/14	4/37	NS*
CT score (Lund-Mackay)	0 (0-2)	6 (2-16)	14 (2-24)	.000† .000‡ .000§
Polyp score (Davos)	0	0	1.5 (0.5-3)	.000† .000§
IL-5 positive				
No. of subjects	0	4	24	.042*† .000‡ .029§

Data are expressed as medians and ranges. The level of significance (*P* value) was obtained by using ANOVA or the Fisher exact test (as marked with an asterisk). For comparisons of variables between 2 groups, the Mann-Whitney *U* test was performed. CT, Computed tomographic; NS, not significant.

*A *P* value of less than .05 was considered statistically significant.

†Control subjects versus patients with CRSsNP.

‡Control subjects versus patients with CRSwNP.

§Patients with CRSsNP versus patients with CRSwNP.

TABLE E3. Detection limits for commercially available cytokine kits

Cytokines	Detection limit (pg/mL)	Source	Methods
IL-5	0.21	R&D Systems	Luminex
ECP	2000	Phadia Diagnostics	ImmunoCAP
Eotaxin	8.2	R&D Systems	Luminex
Periostin	187	R&D Systems	ELISA
IL-4	0.57	R&D Systems	Luminex
IL-13	0.22	Bio-Rad Laboratories	Luminex
TGF- β 1	7.8	R&D Systems	ELISA
IFN- γ	7.8	R&D Systems	ELISA
IL-17	0.39	R&D Systems	Luminex
Fibronectin	156	ALPCO	ELISA
Tenascin C	31.2	US Biological	ELISA