

Periostin May Play a Protective Role in the Development of Eosinophilic Chronic Rhinosinusitis With Nasal Polyps in a Mouse Model

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Objectives/Hypothesis: Several genes have been reported to be upregulated in human nasal polyps in previous genetic analyses. Among these genes, periostin is known to be overexpressed in nasal polyps obtained from aspirin-sensitive patients. Using periostin-null mice, we investigated the role of periostin in a murine model of eosinophilic rhinosinusitis with nasal polyps.

Study Design: Animal study.

Methods: Eosinophilic rhinosinusitis was induced in both periostin-null and wild-type mice according to previously established protocols. In brief, ovalbumin (OVA) was used for sensitization and prolonged intranasal stimulation. *Staphylococcus aureus* enterotoxin B was applied intranasally to develop polyplike lesions. To examine the inflammation and mucosal lesions, hematoxylin and eosin, Sirius red, and Giemsa staining were performed.

Results: There was no definite difference in the maximal mucosal thickness between periostin-null and wild-type mice. In contrast, some parameters of inflammation, including the number of polyplike lesions and mast cells, were aggravated in the periostin-null mice compared to wild type. Eosinophilic infiltration was aggravated in the OVA-stimulated periostin-null mice, compared to OVA-stimulated wild-type mice, whereas there was no apparent difference between wild-type and periostin-null mice challenged with additional *S aureus* enterotoxin B.

Conclusions: The loss of periostin appears to enhance polyplike lesion formation and mast cell infiltration in a mouse model of eosinophilic rhinosinusitis with nasal polyps.

Key Words: Periostin, sinusitis, nasal polyps, mice, eosinophils, mast cells.

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INTRODUCTION

Chronic rhinosinusitis (CRS) with nasal polyps (CRSwNP) impairs the quality of life of affected persons and is associated with substantial medical costs.¹ Although numerous studies have examined the pathogenesis of CRSwNP, it remains poorly understood.² Some studies have compared the level of genetic expression in polyp tissue from patients with CRSwNP with that in the nasal/sinus mucosa of patients with rhinitis or CRS without nasal polyps or normal subjects.^{3–6} In such stud-

ies, genes found to be up- or downregulated in nasal polyps were generally validated by real-time quantitative reverse transcription-polymerase chain reaction (PCR) and immunohistochemistry. Although some distinct findings were obtained, such as the overexpression of mammaglobin and underexpression of the serine protease inhibitor Kazal type 5, the exact role of these proteins has not yet been determined.^{3,6}

A few studies have examined the differential expression of genes or proteins in polyp tissue with respect to aspirin intolerance.^{7,8} In polyps from aspirin-sensitive patients relative to the sinonasal mucosa from normal subjects, gene expression of periostin was found to be upregulated.⁷ In another study, using a protein microarray and Western blot analysis, β -adaptin and heat shock protein 70 were upregulated in polyp tissue from aspirin-sensitive patients compared with that from aspirin-tolerant patients.⁸ The nasal polyps in aspirin-sensitive patients are typically eosinophilic, although no definite difference in histologic appearance has been found in polyps between subjects with and without aspirin intolerance.^{9,10}

A murine model of eosinophilic CRSwNP was established recently.¹¹ By applying similar protocols in periostin-null (PN) mice, we investigated the role of periostin in the development of eosinophilic CRSwNP.

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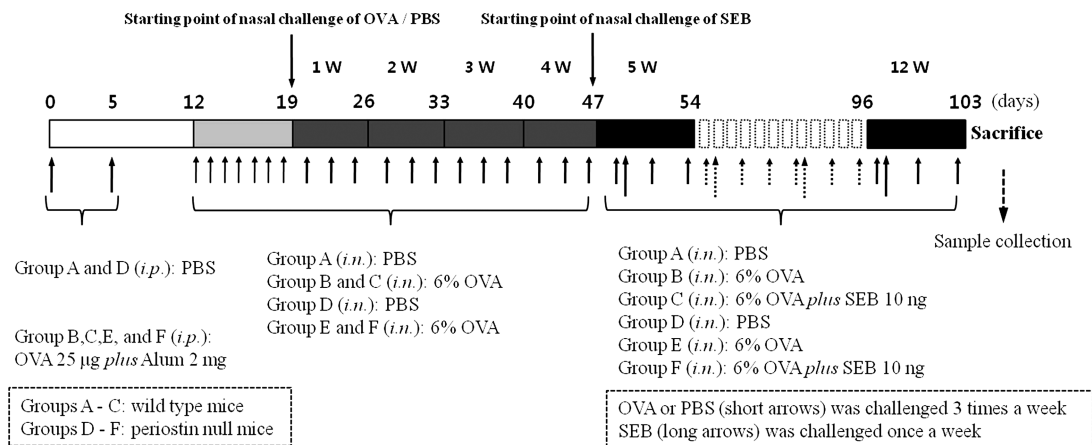


Fig. 1. Protocols for the development of eosinophilic rhinosinusitis with nasal polyps in mice. Ovalbumin (OVA) was used to obtain systemic sensitization and local stimulation in experimental groups B, C, E, and F. Phosphate-buffered saline (PBS) was used for both systemic and local stimulation in the control groups (A and D). *Staphylococcus aureus* enterotoxin B (SEB) diluted in PBS was used as a challenge immediately after the instillation of OVA weekly during the last 8 consecutive weeks in groups C and F. Groups A–C, WT mice; groups D–F, PN mice. *i.p.* = intraperitoneal injection; *i.n.* = intranasal instillation.

MATERIALS AND METHODS

Animals

B6;129-*Postn*^{tm1Jmol/J} mice were purchased from the Jackson Laboratory (Bar Harbor, ME). PN offspring were generated by mating female heterozygotes and male homozygotes of the B6;129-*Postn*^{tm1Jmol/J} mice. Genotyping was done by PCR in accordance with the recommendations of the Jackson Laboratory. Wild-type (WT) C57BL/6J mice were obtained from Koatech Laboratory Animal, Inc. (Pyeongtaek, Korea). The mice were maintained under specific pathogen-free conditions with a 12/12-hour light/dark cycle. Four-week-old PN and WT mice were used in all experiments. This study was approved by the Animal Research Ethics Board of Gyeongsang National University.

Experimental Protocols

The protocols were as described previously, except for the concentration of ovalbumin (OVA) (Grade V; Sigma, St. Louis, MO) (Fig. 1).¹¹ Because the degree of inflammation in a preliminary study was milder in C57BL/6J mice than in the BALB/c mice used in our previous study, the concentration of OVA was doubled.

The mice were categorized into three groups each for WT (groups A, B, and C) and PN (groups D, E, and F). In the control groups (A and D), phosphate-buffered saline (PBS) was applied for both systemic and local stimulation (each group, *n* = 5). The mice in the experimental groups (B, C, E, and F) were systemically sensitized with 25 µg of OVA dissolved in 300 µL of PBS in the presence of 2 mg of aluminum hydroxide gel adjuvant by intraperitoneal injection on days 0 and 5. One week after the second intraperitoneal injection, the mice were challenged intranasally with 6% OVA diluted in 40 µL of PBS. Next, continual local stimulation was maintained in the same fashion three times per week for 12 consecutive weeks (group B, *n* = 5; group E, *n* = 8). In groups C and F, 10 ng of *Staphylococcus aureus* enterotoxin B (SEB) diluted in 20 µL of PBS was used as a challenge immediately after the instillation of OVA weekly during the last 8 consecutive weeks (each group, *n* = 10). At 24 hours after the final nasal challenge, the mice were euthanized and decapitated for histologic analysis.

Histologic Analysis

The heads of the mice were immediately fixed in 2% paraformaldehyde and decalcified in 5% nitric acid for 4 to 5 days at

4°C. Specimens were excised from the second palatal ridge to the first upper molars. The tissue was dehydrated and processed according to standard paraffin wax-embedding procedures. The tissue was cut in coronal sections at 4 µm of thickness. To characterize the inflammatory cells, several stains were used, including hematoxylin and eosin (H&E) for overall inflammation, Sirius red for eosinophils, and Giemsa for mast cells. An atlas of normal murine sinonasal anatomy was used to standardize the anatomic locations being examined.¹² Following the identification of the vomeronasal organ, the superior and inferior maxillary turbinates were identified. The true maxillary sinus and ethmoidal labyrinths were identified at the lesions posterior to the two maxillary turbinates. Two coronal sections with similar sinus cavities were chosen and examined by an independent researcher blinded to the experimental groups. The numbers of eosinophils, mast cells, and polyplike mucosal lesions were counted under 10 high-power fields (×40), and the average value from four different areas was used for between-group comparisons. The maximal mucosal thickness was measured at the transition zone of the olfactory and respiratory epithelia using an image analysis system (NIS-Elements BR 3.0 system; Nikon Eclipse, Tokyo, Japan). Polyplike lesions were defined as distinct mucosal bulges with eosinophilic infiltration and/or microcavity formation (Fig. 2A).¹¹ Protruded lesions without eosinophilic infiltration (Fig. 2B) were not regarded as polyplike lesions. Two consecutive slides were reviewed to obviate processing errors and confirm the mucosal lesions.

Statistical Analyses

The Mann-Whitney *U* test was used to compare the mucosal thickness and number of polyplike lesions and inflammatory cells, including eosinophils and mast cells, between the groups. All statistical analyses were conducted using SPSS version 18.0 for Windows (SPSS, Chicago, IL). Graphs were generated with Prism version 5.0 (GraphPad Software, Inc., San Diego, CA). *P* < .05 was considered to indicate statistical significance.

RESULTS

Expression and Localization of Periostin

Immunohistochemical staining was performed to examine the expression and localization of periostin in

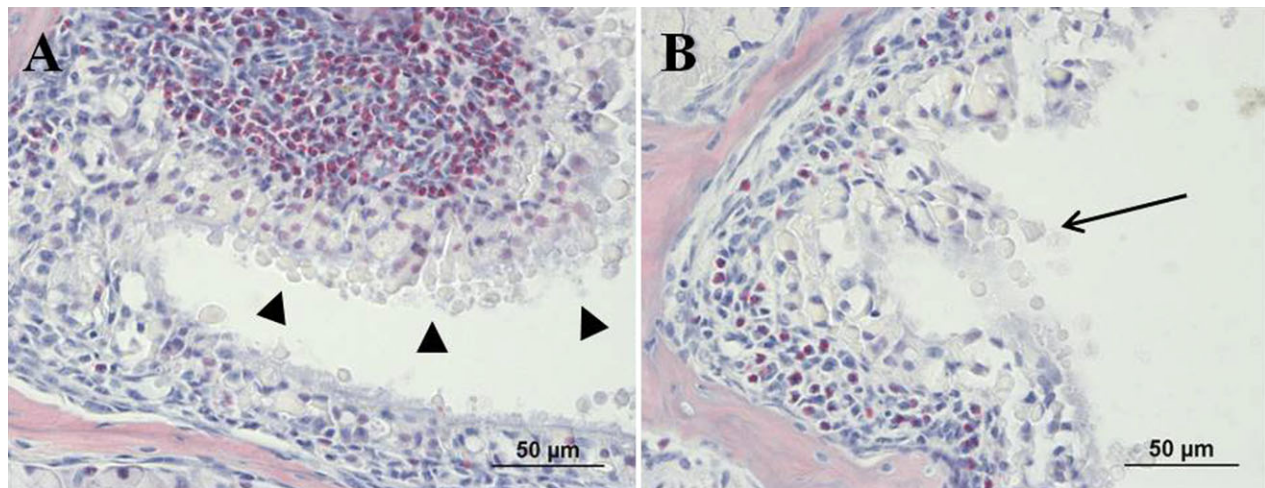


Fig. 2. An example of polyplike lesion. (A) Distinct mucosal bulges with eosinophilic infiltration (arrowheads) were counted as polyplike lesions. (B) Simple protruding lesions without eosinophilic infiltration (arrow) were excluded. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the nasal mucosa in each group. Periostin, which was not expressed in the PN mice, was distributed mainly in the subepithelial tissues including the periosteum of the WT mice. The mucosal thickness and degree of inflammation were apparently different in each group: most severe in group C, moderate in group B, and smallest in group A. In addition, the degree of periostin expression was strongest in group C, followed by groups B and A. (Fig. 3).

Analysis of Mucosal Lesions and Inflammatory Cells

A thickened mucosa with polyplike lesions was observed primarily at the transition zone of the olfactory and respiratory epithelia. There was an apparent difference in the maximal mucosal thickness between the control and experimental groups, regardless of the type of mouse ($P < .01$). Groups C and F demonstrated the

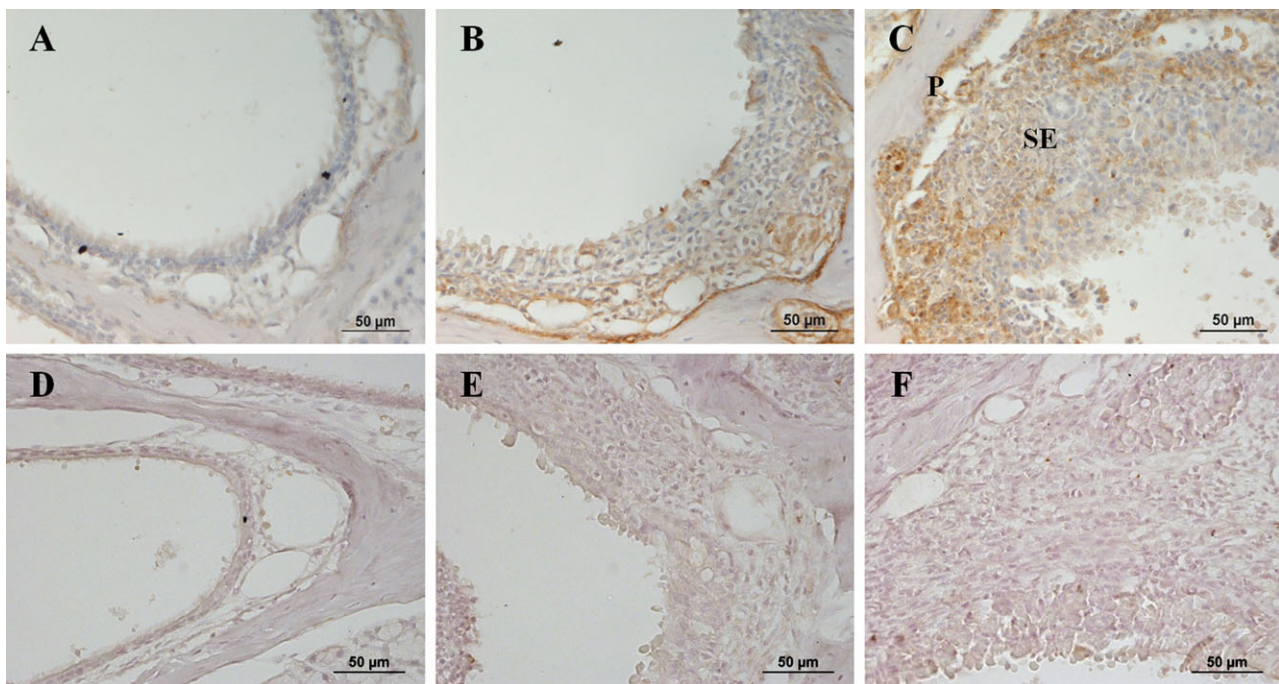


Fig. 3. Immunohistochemistry for periostin. In wild-type mice (groups A–C), periostin was distributed primarily in the subepithelial tissues (SE) including the periosteum (P). The expression of periostin was strongest in group C, moderate in group B, and smallest in group A. In contrast, a lack of periostin expression was confirmed in the periostin-null mice (groups D–F). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

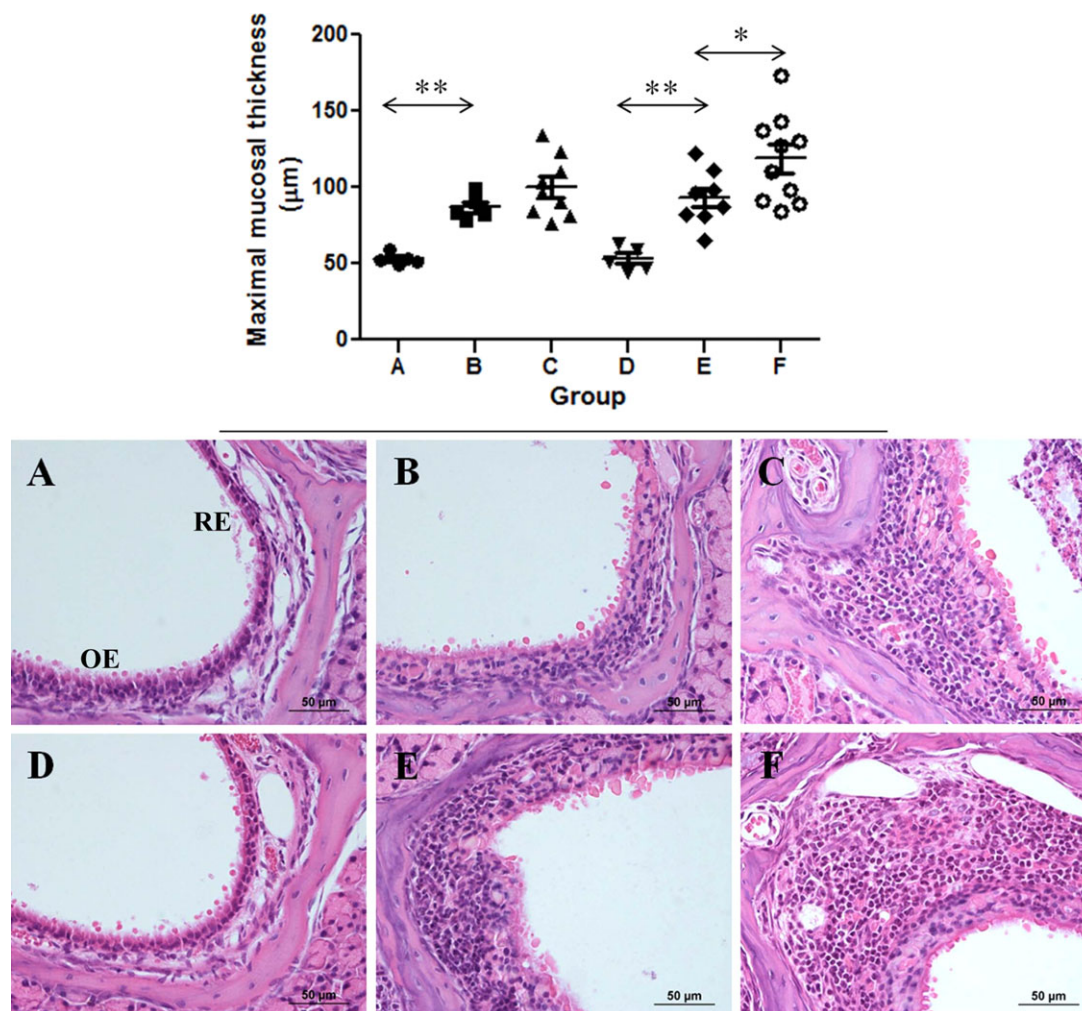


Fig. 4. Comparison of the maximal mucosal thickness between groups. A thickened mucosa with polyplike lesions was observed primarily at the transition zone of the olfactory (OE) and respiratory epithelia (RE). There was an apparent difference in the maximum mucosal thickness between the control and experimental groups in both wild-type and periostin-null mice. In particular, group F showed significantly greater mucosal thickening than group E, and there was no definite difference between groups B and C (* $P < .05$; ** $P < .01$; hematoxylin and eosin staining, $\times 400$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

thickest mucosal layer in the WT and PN mice, respectively. Group F, in particular, showed a significantly thicker mucosa than group E ($P = .04$), whereas there was no clear difference between groups B and C ($P = .23$). There was no definite difference between the WT and PN mice (Fig. 4). Eosinophils accounted for the majority of all inflammatory cells, and the mice in group E had a propensity for more severe eosinophilic infiltration than those in group B ($P = .04$). However, no significant difference was found in the eosinophil count between groups C and F (Fig. 5).

With regard to polyplike lesion formation, there were no definite polyplike lesions in group B or E, although both groups showed definite mucosal thickening accompanied by inflammation compared with the control groups. Polyplike lesions were found only in the mice that received SEB intranasally (groups C and F). In addition, group F showed significantly larger numbers of polyplike lesions than group C ($P = .01$; Fig. 6).

Mast cells were identified by their characteristic cytoplasmic granules using Giemsa staining. Groups E and F showed a markedly denser distribution of mast cells than groups B and C ($P = .03$ and $P < .01$, respectively). Furthermore, group F displayed more pronounced mast cell infiltration than group E ($P = .03$; Fig. 7).

DISCUSSION

Periostin, a component of the extracellular matrix, was identified in the periosteum and periodontal ligament in adult mice and was presumed to play a role in the recruitment and attachment of osteoblast precursors in the periosteum.¹³ Periostin expression was increased by transforming growth factor- β , which has dramatic effects on periosteal expansion. Thus, PN mice were generated to examine the function of periostin; severe growth retardation and incisor enamel defects were seen as a consequence.¹⁴ Periostin is also known to be involved in heart morphogenesis, wound repair, and the development

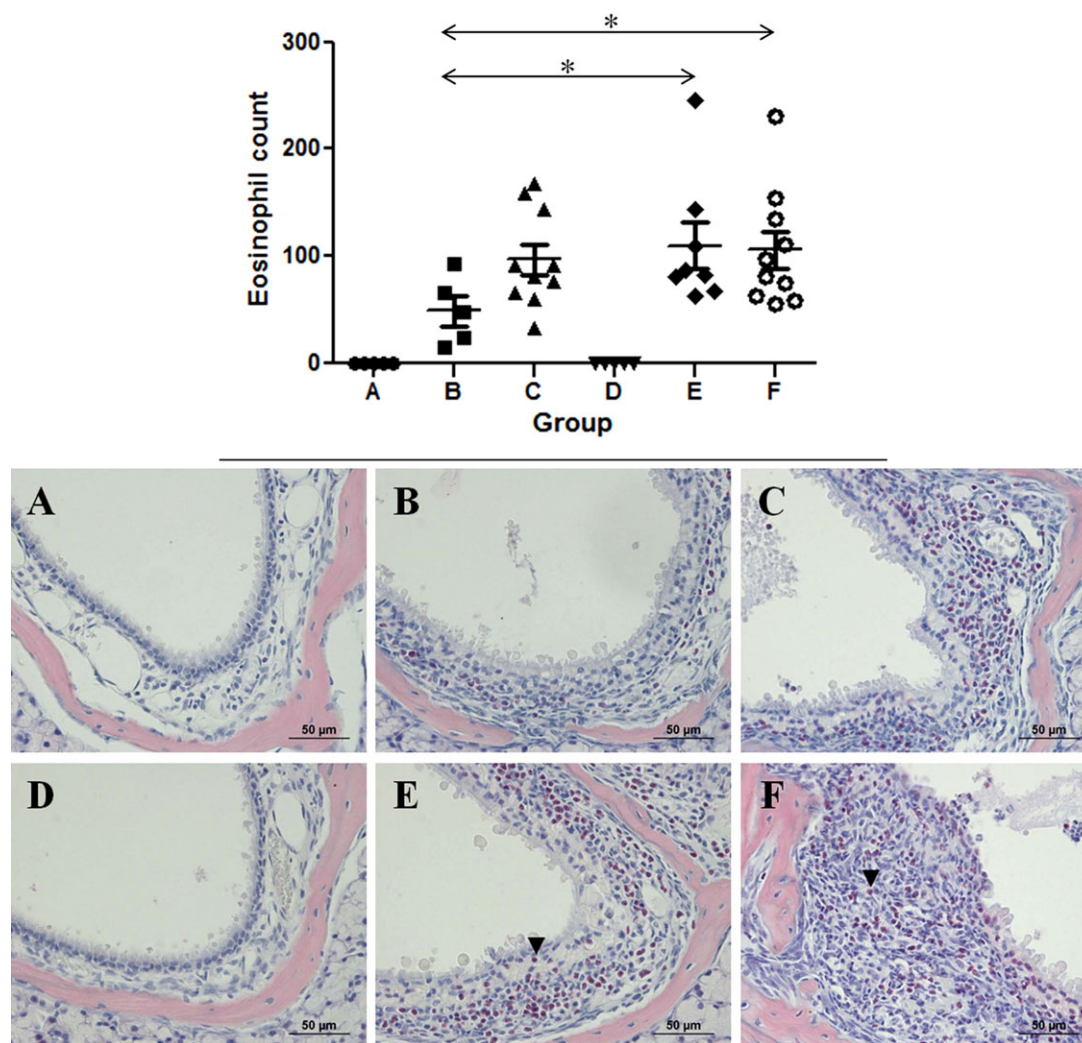


Fig. 5. Comparison of the eosinophil count between groups. The mice in group E showed more pronounced eosinophilic infiltration (▼) than those in group B. However, no significant difference was found in the eosinophil count between groups C and F ($*P < .05$; Sirius red staining, $\times 400$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of various tumors.^{15–17} In mice, it is expressed in embryonic and fetal heart, specifically in the endocardial

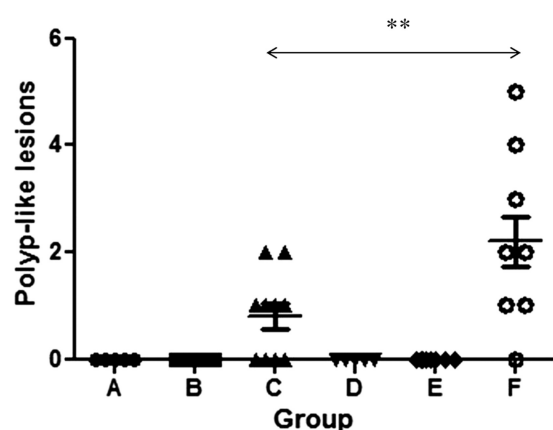


Fig. 6. Comparison of polyplike lesion counts between groups. Polyplike lesions were found only in groups C and F. The number of polyplike lesions was larger in group F than in group C ($**P < .01$).

cushions, and PN mice exhibit atrial septal defects and structural abnormalities in their atrioventricular valves.^{18,19} In breast cancer patients with bone metastases, significantly elevated serum periostin levels have been identified; in patients with non-small cell lung cancer, a positive correlation was found between the expression of periostin and tumor size/disease stage/metastasis.^{20,21}

On the other hand, some studies have examined the role of periostin in eosinophil-related inflammatory diseases, including bronchial asthma and eosinophilic esophagitis.^{22–24} Periostin was highly expressed and localized specifically in the subepithelial areas of bronchial tissues not only in patients with asthma, but also in OVA-sensitized and OVA-inhaled mice. Subepithelial fibrosis caused by the secretion of periostin was found to be controlled by IL-4 and/or IL-13 using IL-4 or IL-13 knockout mice.²² The overexpression of periostin was also demonstrated in esophageal tissues obtained from patients with eosinophilic esophagitis, compared with control subjects. The role of periostin in eosinophil recruitment was also investigated using mice sensitized with an *Aspergillus*

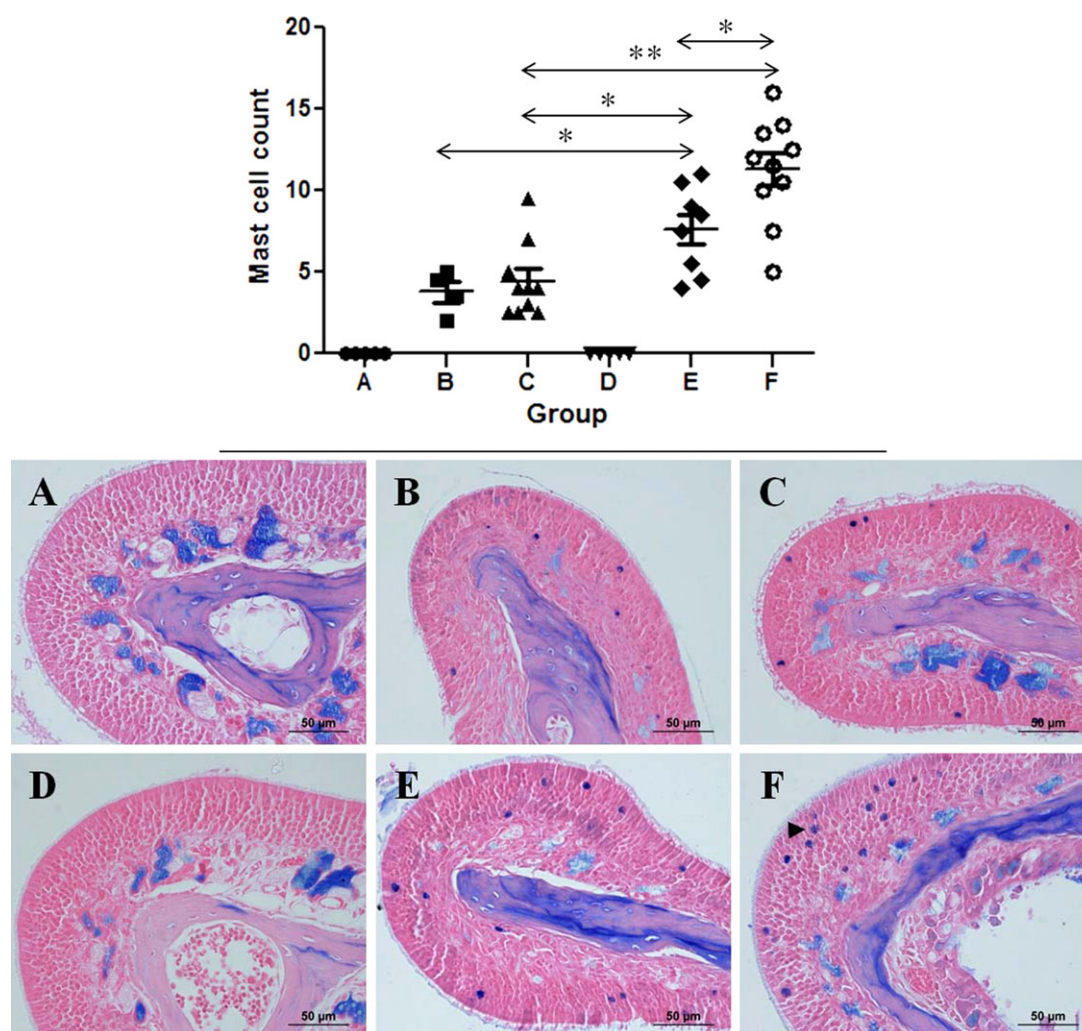


Fig. 7. Comparison of mast cell counts between groups. Groups E and F showed a denser distribution of mast cells (►) than groups B and C. The difference was also significant between groups E and F (* $P < .05$; ** $P < .01$; Giemsa staining, $\times 400$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fumigates extract. Interestingly, eosinophilic infiltration into tissues was significantly lower in periostin-deficient mice compared with WT mice, although the blood eosinophil count was higher. It was concluded that periostin may regulate eosinophil accumulation in tissues.²³ In contrast, in the present study, tissue eosinophilia was more prominent in PN mice (group E) than in WT mice that were stimulated with OVA (group B), although the difference was not apparent in the presence of additional SEB, that is, no significant difference between groups C and F. In addition, the number of polyplike lesions and the mast cell count were larger in PN mice than in WT mice. The differences between the former and our studies might be due to organ specificity, although further study is needed to confirm it. Our results are consistent with increased airway hyperresponsiveness in PN mice, which was reported in a recent study using a mouse model of allergic lung disease.²⁴ Airway hyperresponsiveness and elevated serum IgE levels were noted in PN mice, whereas mucus metaplasia and peribronchial fibrosis did not differ between PN and WT mice. Similarly, in the current study, there was no

definite difference in the mucosal thickness, but there were some differences in the degree of inflammation such as mast cell infiltration and formation of polyplike lesions between PN and WT mice. Accordingly, given the concept of combined airways, periostin appears to exert anti-inflammatory actions in eosinophilic or allergic airway diseases.²⁵ There are some limitations in this study. First, the comparison between groups was mainly based on the cell count. We did not prove the difference between PN and WT mice using more quantitative methods such as a quantitative real-time PCR for inflammatory cytokines including interferon- γ , interleukin (IL)-4, and IL-5, which could have supported our hypothesis on the role of periostin. Second, the degree of inflammation in each murine nasal mucosa can be influenced by some factors such as individual reactivity to OVA and/or SEB in the presence or absence of periostin, which possibly confounded the results. Despite these limitations, in the present study, it was ascertained that the loss of periostin may aggravate the tissue inflammation and formation of mucosal lesions in a mouse model of eosinophilic CRSwNP.

CONCLUSION

This study demonstrated that the loss of periostin may enhance the recruitment of mast cells and induce more polypoid lesions in a mouse model. Its effect on eosinophils, however, was inconclusive. Further studies, including human studies, will be needed to clarify the role of periostin.

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