

Letter to the Editor

Periostin upregulates the effector functions of eosinophils

To the Editor:

Periostin, an extracellular matrix (ECM) protein, has recently been highlighted as a biomarker of T_H2 immune response in bronchial asthma.¹ Periostin expression is upregulated in bronchial epithelial cells and fibroblasts of those with asthma, and its concentration in serum is increased in asthma.^{2,3} Indeed, Jia et al² identified serum periostin as the single best predictor of airway eosinophilia in patients with severe asthma. However, the role of periostin in the development of allergic inflammation has not been fully clarified. The effect of periostin deletion on allergic inflammation in mouse models is still controversial.^{1,4}

Periostin functions as a matricellular protein^{1,4}; that is, it is an ECM protein that binds to cellular receptors and functions in cell activation. Several integrins such as $\alpha V\beta 1$ and $\alpha V\beta 3$ have been reported as receptors for periostin,^{1,4} although little is known about the periostin receptors on eosinophils. Johansson et al⁵ reported that periostin increased eosinophil adhesion through the $\alpha M\beta 2$ integrin. However, the effects of periostin on other functions of eosinophils such as superoxide anion (O_2^-) generation, degranulation, or the production of cytokines such as TGF- β have not been examined.

We first examined whether periostin induces the adhesion of eosinophils in our experimental system. Eosinophils were obtained from healthy volunteers or allergic subjects with allergic rhinitis, and their adhesion to periostin or vascular cell adhesion molecule (VCAM)-1 was measured using residual eosinophil peroxidase assays (see this article's [Methods](#) section in the Online Repository at www.jacionline.org). Compared with control, periostin induced significantly higher adhesion of blood eosinophils isolated from healthy volunteers, to a level similar to that induced by VCAM-1 (Fig 1, A). Furthermore, IL-5 upregulated blood eosinophil adhesion to periostin, but not to VCAM-1 (Fig 1, A). In allergic subjects, periostin or VCAM-1 also enhanced the adhesion of eosinophils versus control (Fig 1, B), and adhesion induced by both molecules was higher than that of healthy volunteers (Fig 1, B). Adhesion of eosinophils from healthy volunteers was further characterized. Eosinophil adhesion to periostin was greater than that to intercellular adhesion molecule 1 (Fig 1, C). Furthermore, periostin-induced adhesion of eosinophils was inhibited by anti- αM or anti- $\beta 2$ integrin antibody but not by anti- $\alpha 4$ integrin mAb (Fig 1, D). Anti- αM or anti- $\beta 2$ integrin antibody suppressed eosinophil adhesion to a greater extent when we used higher concentrations of periostin or antibodies (see Fig E2 in this article's Online Repository at www.jacionline.org). Periostin induced significantly higher eosinophil O_2^- generation, to a level similar to that induced by IL-5 (Fig 1, E; see Fig E3 in this article's Online Repository at www.jacionline.org). Eosinophil O_2^- generation induced by periostin was suppressed by anti- αM or anti- $\beta 2$ integrin mAb but not by anti- $\alpha 4$ integrin mAb (Fig 1, F).

Next, we examined the effect of periostin on the degranulation of eosinophils. Compared with control, periostin induced

significantly higher eosinophil-derived neurotoxin (EDN) release, to a level similar to that induced by IL-5 (Fig 2, A), and this release was suppressed by anti- αM or anti- $\beta 2$ integrin mAb (Fig 2, B). Furthermore, periostin increased the production of cytokines and chemokines such as IL-6 and IL-8 from eosinophils (see Table E1 in this article's Online Repository at www.jacionline.org). Periostin also increased the production of TGF- $\beta 1$, TGF- $\beta 2$, cysteinyl leukotrienes (cysLTs), and prostaglandin E_2 from eosinophils (Fig 2, C-F). Concentrations of T_H2 cytokines such as IL-4 or IL-13 were below the detection limit (data not shown).

In most types of cells, αV integrins mediate adhesion to periostin and cell activation.^{1,4} However, the integrins involved in periostin-induced cell activation differ between cell types. Eosinophils lack αV integrins but possess a unique repertoire of 7 integrins: $\alpha 4\beta 1$, $\alpha 6\beta 1$, $\alpha L\beta 2$, $\alpha M\beta 2$, $\alpha X\beta 2$, $\alpha D\beta 2$, and $\alpha 4\beta 7$ integrins.^{5,6} Johansson et al⁵ reported that periostin increases eosinophil adhesion and that this increase is mediated by $\alpha M\beta 2$ integrin,⁵ which is consistent with our findings (Figs 1 and 2). Furthermore, we found that periostin activated eosinophil functions such as O_2^- generation and EDN release, and that their induction by periostin was suppressed by anti- αM or anti- $\beta 2$ integrin mAb. The combined data suggested that periostin activates eosinophil functions through the $\alpha M\beta 2$ integrin, which could possibly aggravate asthma clinically. As for airway eosinophils, these eosinophils have highly activated $\alpha M\beta 2$ integrin.⁶ The report that anti-IL-5 antibody suppresses the expression of $\beta 2$ integrin on blood eosinophils, but not on airway eosinophils,⁷ indicates important roles of mediators other than IL-5, such as periostin, in the upregulation of $\beta 2$ integrins and eosinophil functions in the airway.

Periostin likely plays an important role in the process of tissue remodeling. Subepithelial fibrosis, a cardinal feature of bronchial asthma, is thickening of the lamina reticularis, in which collagens I, III, and V, fibronectin, and tenascin-C are deposited beneath the basal lamina.¹ The localization of periostin is the same as that of these ECM proteins, suggesting that periostin is a novel component of subepithelial fibrosis. In the present study, periostin increased TGF- $\beta 1$, TGF- $\beta 2$, cysLTs, or prostaglandin E_2 production from eosinophils (Fig 2), which could promote airway remodeling in asthma. TGF- β increases the expression of periostin in fibroblasts,⁸ which may further activate eosinophil functions. Furthermore, periostin derived from epithelial cells upregulates TGF- β production and activation in epithelial cells through a matrix metalloproteinase-dependent mechanism.⁹ Therefore, periostin is not only a novel element of tissue remodeling but also interacts with other cells such as eosinophils to promote remodeling. Increased periostin is associated with greater airflow limitation in patients with asthma receiving inhaled corticosteroid (ICS),³ which is consistent with the idea that periostin is involved in the development of airway remodeling.

It was suggested that periostin is a marker for corticosteroid insensitivity. Periostin expression is increased even with maximal ICS treatment.^{2,3,10} Matsumoto¹⁰ reported that ICS treatment decreased serum periostin levels only by 5.4% from baseline, although it decreased fractional exhaled nitric oxide

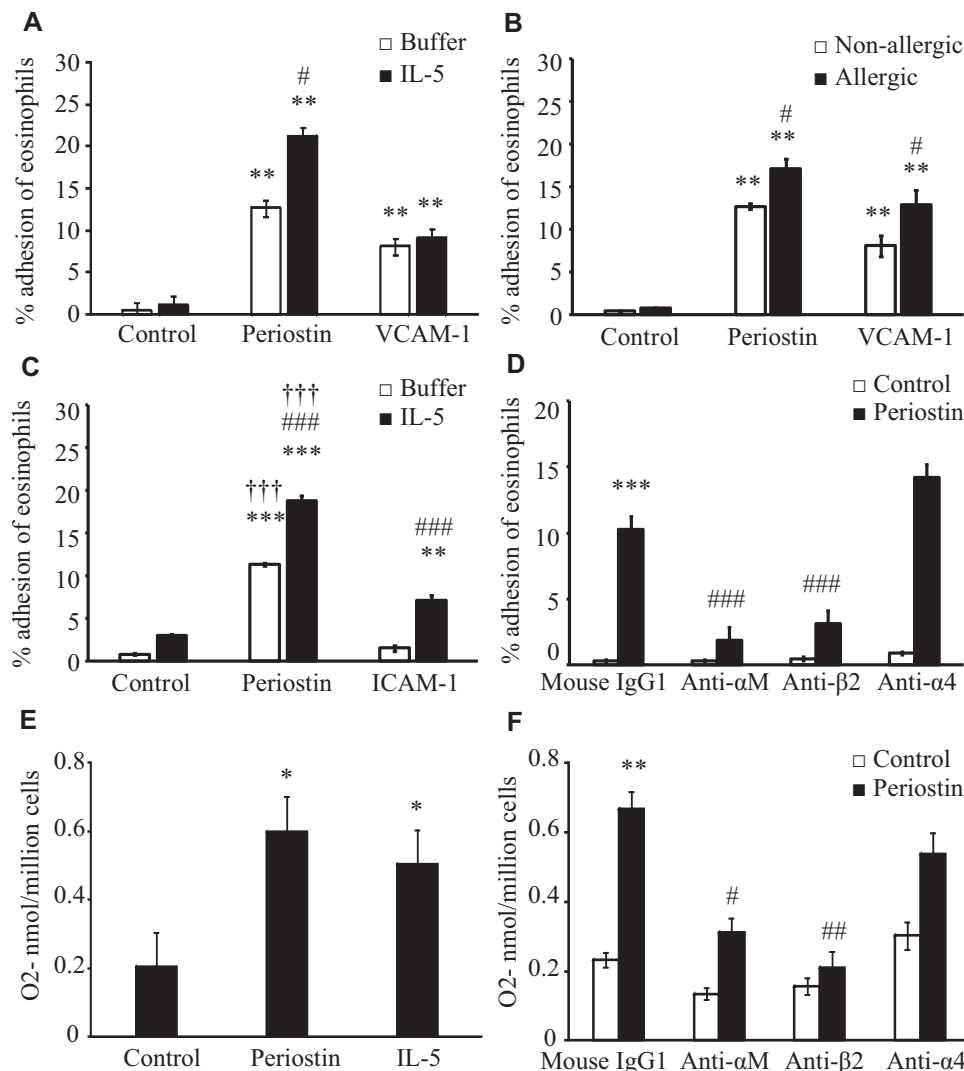


FIG 1. Effect of periostin on eosinophil adhesion or eosinophil O₂⁻ generation. **A**, Effect of periostin on adhesion of eosinophils. Eosinophils (1×10^5 cells/mL) from nonallergic donors were incubated with or without IL-5 (100 pM), and their adhesion to periostin (10 μ g/mL) and VCAM-1 (5 μ g/mL) was measured. Data are shown as means $\pm$ SEM of 7 experiments using cells from different donors. ** $P < .01$ versus control. # $P < .05$ versus buffer (without IL-5). **B**, Eosinophils were collected from nonallergic or allergic donors, and their adhesion to periostin (10 μ g/mL) or VCAM-1 (5 μ g/mL) was measured ($n = 7$). ** $P < .01$ versus control. # $P < .05$ versus nonallergic donors. **C**, Eosinophils were collected from nonallergic donors, and their adhesion to periostin (10 μ g/mL) or recombinant human-ICAM-1 (10 μ g/mL) was measured ($n = 6$). ** $P < .01$ and *** $P < .001$ versus control. ### $P < .001$ versus buffer (without IL-5). ††† $P < .001$ versus ICAM-1. **D**, Effect of anti-integrin mAbs on eosinophil adhesion to periostin. Eosinophils from nonallergic donors were preincubated with mouse IgG₁, anti-αM integrin mAb, anti-β2 integrin mAb, or anti-α4 integrin mAb (3 μ g/mL, each) for 15 minutes. Adhesion of the eosinophils to periostin was then assessed ($n = 6$). *** $P < .001$ versus control. ### $P < .001$ versus mouse IgG₁. **E**, Effect of periostin on eosinophil O₂⁻ generation. Eosinophils (1×10^6 cells/mL) from nonallergic donors were incubated in the presence or absence of IL-5 (100 pM) in periostin-coated plates (10 μ g/mL) or -uncoated plates. The generation of eosinophil O₂⁻ was examined ($n = 6$). The maximum value of eosinophil O₂⁻ generation is shown. * $P < .05$ versus control. **F**, Effect of anti-integrin mAbs on eosinophil O₂⁻ generation induced by periostin. Eosinophils were preincubated with mouse IgG₁, anti-β2 integrin mAb, anti-αM integrin mAb, or anti-α4 integrin mAb (3 μ g/mL each) for 15 minutes, and were then added to periostin-coated plates (10 μ g/mL). The generation of eosinophil O₂⁻ was examined ($n = 6$). ** $P < .01$ versus control. # $P < .05$ and ## $P < .01$ versus mouse IgG₁. ICAM, Intercellular adhesion molecule.

levels by 27.4%. In an *in vitro* study, dexamethasone did not suppress TGF-β-induced production of periostin by fibroblasts,⁸ although it did suppress IL-4/IL-13-induced production of periostin. Therefore, TGF-β-induced periostin would play a role in the development of corticosteroid insensitivity, and

interaction between eosinophils and periostin may be involved in this process.

The limitation of this study is that we only conducted an *in vitro* study. Whether interaction between eosinophils and periostin actually occurs in blood or airways in patients with

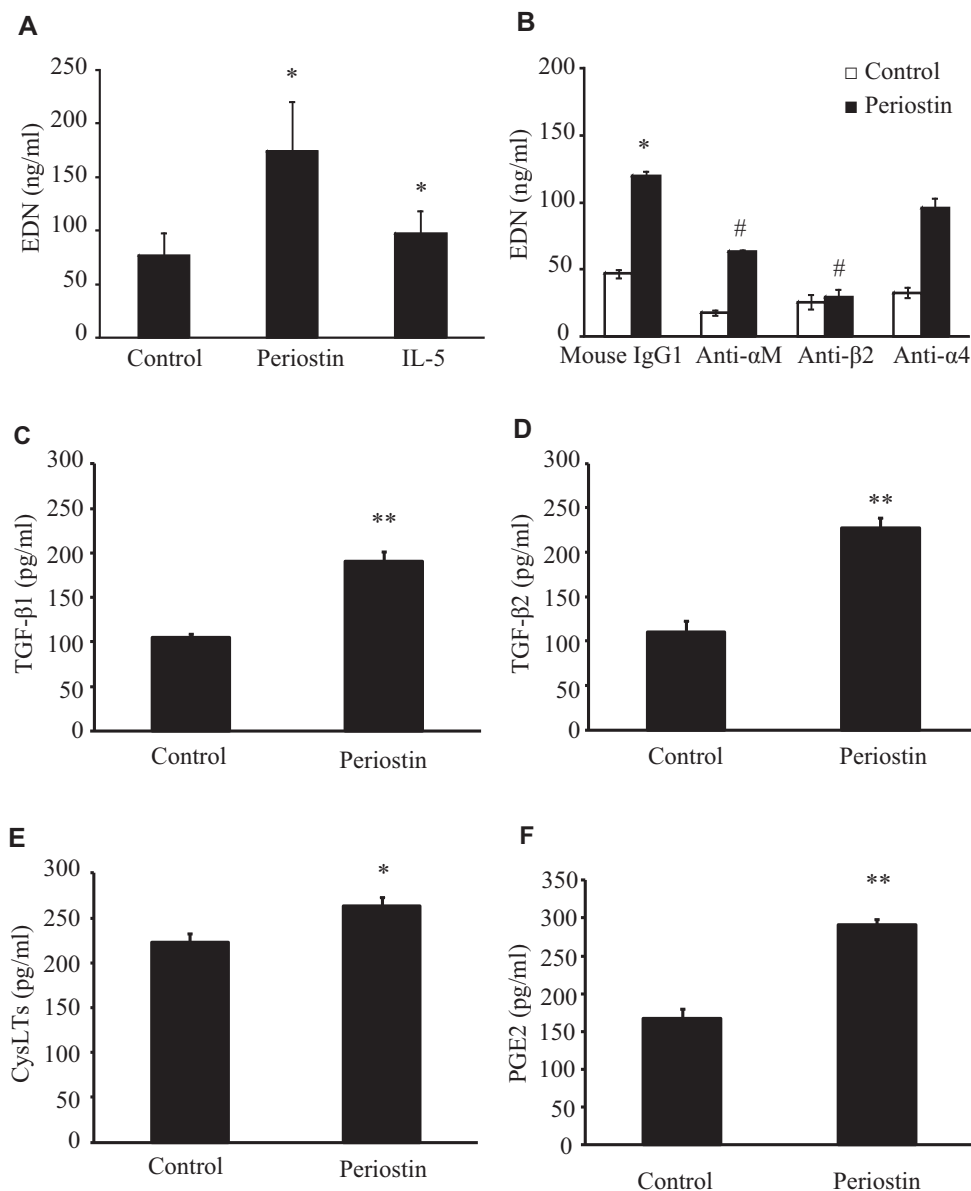


FIG 2. Effect of periostin on EDN release or production of cytokines. **A**, Effect of periostin on EDN release. Eosinophils (1×10^6 cells/mL) from nonallergic donors were incubated in the presence or absence of periostin or IL-5 for 240 minutes. Levels of EDN in cell-free supernatants were quantified using ELISA ($n = 6$). * $P < .05$ versus control. **B**, Effect of anti-integrin mAbs on EDN release induced by periostin. Eosinophils were preincubated with mouse IgG₁, or with anti-β2, anti-αM, or anti-α4 integrin mAbs (3 μg/mL each) for 15 minutes, and were then incubated for 240 minutes with periostin. Levels of EDN in supernatants were quantified ($n = 6$). * $P < .05$ versus control. # $P < .05$ versus mouse IgG₁. **C-F**, Effect of periostin on TGF-β1, TGF-β2, cysLTs, or PGE₂ production. Following separation, eosinophils (1×10^6 cells/mL) from nonallergic donors were resuspended in RPMI 1640 with 2% BSA (for measurement of TGF-β) or with 10% FBS (for measurement of cysLTs or PGE₂) and were incubated with periostin for 24 hours. Concentrations of TGF-β1 (Fig 2, C), TGF-β2 (Fig 2, D), cysLTs (Fig 2, E), or PGE₂ (Fig 2, F) in the supernatants were measured by ELISA ($n = 6$). * $P < .05$ and ** $P < .01$ versus control. PGE₂, Prostaglandin E₂.

asthma is still unknown because the concentration of periostin that we coated on the plates was much higher than that in serum and bronchoalveolar lavage fluid.^{3,10} However, we postulate that periostin could directly activate eosinophils in some situations, probably in patients with T_H2-high asthma or with airway remodeling.

Here, we report that periostin upregulates eosinophil functions such as O₂⁻ generation and EDN release through αMβ2 integrin. Furthermore, periostin increases the production of TGF-β and cysLTs from eosinophils. These effects may be involved in the activation of eosinophils in the airway of T_H2-dominant asthma, and promote airway remodeling.

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REFERENCES

- Izuhara K, Arima K, Ohta S, Suzuki S, Inamitsu M, Yamamoto K. Periostin in allergic inflammation. *Allergol Int* 2014;63:143-51.
- Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma (BOBCAT) Study Group. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012;130:647-54.
- Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;132:305-12.
- Masuoka M, Shiraishi H, Ohta S, Suzuki S, Arima K, Aoki S, et al. Periostin promotes chronic allergic inflammation in response to Th2 cytokines. *J Clin Invest* 2012;122:2590-600.
- Johansson MW, Annis DS, Mosher DF. $\alpha_M\beta_2$ integrin-mediated adhesion and motility of IL-5-stimulated eosinophils on periostin. *Am J Respir Cell Mol Biol* 2013;48:503-10.
- Johansson MW, Mosher DF. Integrin activation states and eosinophil recruitment in asthma. *Front Pharmacol* 2013;4:33.
- Johansson MW, Gunderson KA, Kelly EA, Denlinger LC, Jarjour NN, Mosher DF. Anti-IL-5 attenuates activation and surface density of $\beta(2)$ -integrins on circulating eosinophils after segmental antigen challenge. *Clin Exp Allergy* 2013;43:292-303.
- Shoda T, Futamura K, Kobayashi F, Saito H, Matsumoto K, Matsuda A. Cell type-dependent effects of corticosteroid on periostin production by primary human tissue cells. *Allergy* 2013;68:1467-70.
- 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.
- Matsumoto H. Serum periostin: a novel biomarker for asthma management. *Allergol Int* 2014;63:153-60.

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METHODS

Preparation of eosinophils

Eosinophils were isolated from peripheral blood collected from nonallergic healthy volunteers with a peripheral blood differential eosinophil count of less than 5%. *Healthy volunteers* were defined as subjects with no history of allergic disease such as asthma or rhinitis and no symptoms such as coughing or sneezing associated with allergic disease. We measured allergen-specific IgE antibodies of some, but not all, subjects and no subject tested had specific IgE antibodies to environmental allergens such as house dust mite, molds, or Japanese cedar pollen. In selected experiments, we collected eosinophils from allergic subjects who have perennial or seasonal allergic rhinitis without taking any medication. We confirmed that all allergic subjects had serum IgE antibodies specific for either house dust mite or Japanese cedar pollen. The number of men and women, ranging in age from 24 to 59 years, was comparable among the donors. This study was approved by the Ethical Committee of Saitama Medical University, and written informed consent was obtained before collection of each blood sample. Eosinophils were isolated by the combination of Percoll density gradient centrifugation and negative selection using immunomagnetic beads, as previously described.^{E1-E6} More than 98% of the cells were eosinophils, as determined by morphological criteria using May-Grünwald-Giemsa staining. Eosinophil viability was more than 99%, as determined by Trypan blue dye exclusion. Eosinophils were resuspended in HBSS supplemented with gelatin (Sigma-Aldrich, St Louis, Mo) to a final concentration of 0.1% (HBSS/gel).

Eosinophil adhesion

Eosinophil adhesion to recombinant human (rh)-periostin, rh-VCAM-1, or rh-intercellular adhesion molecule (ICAM)-1-coated plates was assessed on the basis of residual eosinophil peroxidase activity of adherent eosinophils, as previously described.^{E1-E6} First, rh-periostin (10 μ g/mL), rh-VCAM-1 (5 μ g/mL), or rh-ICAM-1 (10 μ g/mL) (all from R&D Systems, Minneapolis, Minn) was dissolved in 0.05 mol NaHCO₃ coating buffer (15 mM NaHCO₃, 35 mM Na₂CO₃, pH 9.2), was then added to 96-well tissue-culture plates (50 μ L/well; Corning Incorporated, Corning, NY), and incubated at 4°C overnight. The wells were decanted and washed with HBSS, and then 200 μ L of HBSS/gel was added to reduce nonspecific adhesion. After 1-hour incubation at ambient temperature, the wells were decanted and washed. Eosinophils (100 μ L of 1×10^5 cells/mL in HBSS/gel) from nonallergic volunteers or allergic subjects were incubated in the presence or absence of IL-5 (100 pM; R&D Systems) on rh-periostin, rh-VCAM-1, or rh-ICAM-1-coated plates at 37°C for 20 minutes. The plates were washed with HBSS and 100 μ L of HBSS/gel was then added to the wells. Standards that were composed of 100 μ L of serially diluted cell suspensions (1×10^3 , 3×10^3 , 1×10^4 , 3×10^4 , and 1×10^5 cells/mL) were added to the empty wells. The eosinophil peroxidase substrate (1 mM *o*-phenylenediamine, 1 mM H₂O₂, and 0.1% Triton X-100 in Tris buffer, pH 8.0) was then added to all wells and the plates were incubated for 30 minutes at room temperature. The reaction was stopped by adding 20 μ L of 4 mol H₂SO₄ and absorbance was measured at 490 nm. In some experiments, suspended eosinophils were preincubated with 3 μ g/mL of mouse IgG₁ (clone MOPC-21; Becton Dickinson, Franklin Lakes, NJ), anti- α M integrin mAb (clone 2LPM19c; Pierce, Rockford, Ill), anti- β 2 integrin mAb (clone L130; Becton Dickinson), or anti- α 4 integrin mAb (clone HP2/1; Cosmo Bio Co Ltd, Tokyo, Japan) in HBSS/gel for 15 minutes at room temperature before addition to the wells. Each experiment was performed in quadruplicate using eosinophils from a single donor, and the percentage eosinophil adhesion was determined from mean values that were calculated from log dose-response curves. Eosinophil viability after incubation was more than 98%, as determined by Trypan blue dye exclusion.

Eosinophil O₂⁻ generation

Eosinophil O₂⁻ generation was measured in 96-well ELISA plates as previously described on the basis of superoxide dismutase (SOD)-inhibitable reduction of cytochrome C.^{E2-E4} We initially added SOD (0.2 mg/mL in HBSS/gel; 20 μ L) to SOD control wells and then HBSS/gel to all wells of rh-periostin-coated plates (10 μ g/mL) to bring the final volume to 100 μ L. Eosinophil density was adjusted to 1.25×10^6 cells/mL of HBSS/gel mixed

in a 4:1 ratio with cytochrome C (12 mg/mL of HBSS/gel), and 100 μ L of eosinophil suspension was then added to all wells. Immediately after adding IL-5 (100 pM) to the eosinophils, the absorbance of the cell suspensions in the wells was measured at 550 nm in an Immuno-Mini (NJ-2300; Japan Intermed Co, Tokyo, Japan), followed by repeated measurements over the next 240 minutes. In some experiments, suspended eosinophils were preincubated with 3 μ g/mL of mouse IgG₁, anti- α M integrin mAb, anti- β 2 integrin mAb, or anti- α 4 integrin mAb for 15 minutes at room temperature before addition to the wells. Each reaction was evaluated in duplicate against the control reaction in wells containing 20 μ g/mL of SOD. The results were adjusted for a 1-mL reaction volume, and O₂⁻ generation was calculated at an extinction coefficient of 21.1 mM⁻¹ cm⁻¹, as nanomoles of cytochrome C reduced per 1.0×10^6 cells/mL minus the SOD control. The maximum value observed over the incubation time was determined for evaluation of the effects of various factors on eosinophil O₂⁻ generation.

Eosinophil degranulation

Eosinophils (1×10^6 cells/mL) in 96-well plates were incubated for the 240 minutes that were required for measurement of O₂⁻ generation, and were then immediately centrifuged (1500 rpm) at 4°C for 10 minutes. Recovered cell-free supernatants were measured for EDN, as described previously.^{E2-E4} Levels of EDN were quantified using ELISA kits (Medical and Biological Laboratory Co Ltd, Nagoya, Japan).

Cytokine production

Eosinophils (1×10^6 cells/mL) were resuspended in RPMI 1640 with 10% FBS or with 2% BSA (for measurement of TGF- β). The cells were incubated in 96-well periostin-coated tissue-culture plates for 24 hours at 37°C and 5% CO₂.^{E3,E6} After culture, the supernatants were collected, and cytokine and chemokine concentrations were analyzed using Bio-Plex human 27 cytokine assay kits and the Bio-Plex suspension array system (Bio-Rad, Mississauga, Canada). TGF- β 1, TGF- β 2, cysLTs, or prostaglandin E₂ concentrations were measured using ELISA kits (TGF- β 1 and TGF- β 2; R&D Systems, cysLTs or prostaglandin E₂; Cayman Chemical, Ann Arbor, Mich).

The kinetics of eosinophil viability in culture was examined by Trypan blue dye exclusion. The viability of eosinophils after 24-hour culture in our system was about 90% to 95% (Fig E1; 90.6% [without stimulation], 96.0% (+IL-5), or 94.4% [+periostin]). However, the viability after 48-hour culture was much decreased to about 40% to 60% (Fig E1; 46.6% [without stimulation], 66.8% [+IL-5], or 47.0% [+periostin]). Therefore, in this study, we incubated eosinophils for 24 hours for cytokine measurement.

Statistical analysis

Values are expressed as means $\pm$ standard error of the mean. Results were compared using a 1-way factorial ANOVA with Tukey test for multiple comparisons.

P values of less than .05 were considered statistically significant.

REFERENCES

- E1. Nagata M, Saito K, Tsuchiya T, Sakamoto Y. Leukotriene D₄ upregulates eosinophil adhesion via the cysteinyl leukotriene 1 receptor. *J Allergy Clin Immunol* 2002;109:676-80.
- E2. Saito K, Nagata M, Kikuchi I, Sakamoto Y. Leukotriene D₄ and eosinophil trans-endothelial migration, superoxide generation, and degranulation via beta2 integrin. *Ann Allergy Asthma Immunol* 2004;93:594-600.
- E3. Takaku Y, Nakagome K, Kobayashi T, Hagiwara K, Kanazawa M, Nagata M. IFN- γ -inducible protein of 10 kDa upregulates the effector functions of eosinophils through β 2 integrin and CXCR3. *Respir Res* 2011;12:138.
- E4. Noguchi T, Nakagome K, Kobayashi T, Ueda Y, Soma T, Nakamoto H, et al. Effect of beta-2 adrenergic agonists on eosinophil adhesion, superoxide anion generation, and degranulation. *Allergol Int* 2015;64:S46-53.
- E5. Kobayashi T, Takaku Y, Yokote A, Miyazawa H, Soma T, Hagiwara K, et al. Interferon- β augments eosinophil adhesion inducing activity of endothelial cells. *Eur Respir J* 2008;32:1540-7.
- E6. Kobayashi T, Kouzaki H, Kita H. Human eosinophils recognize endogenous danger signal crystalline uric acid and produce proinflammatory cytokines mediated by autocrine ATP. *J Immunol* 2010;184:6350-8.

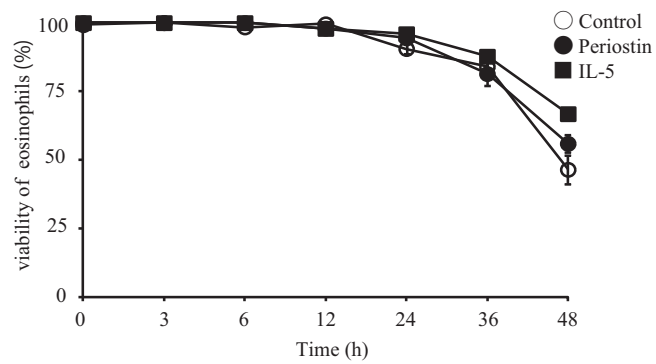


FIG E1. Kinetics of eosinophil viability on incubation with periostin. Eosinophils from nonallergic donors were incubated without stimulation (control), with IL-5 (100 pM), or with periostin (10 μ g/mL). Eosinophil viability was assessed by Trypan blue dye exclusion at the indicated time after stimulation (n = 4).

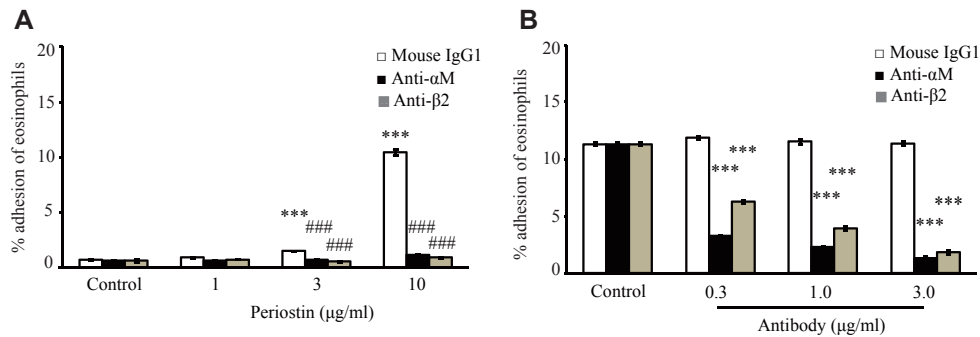


FIG E2. Effect of increased periostin or antibody concentration on the suppression of eosinophil adhesion by anti-αM or anti-β2 integrin antibody. **A**, Effect of periostin concentration on the suppression of eosinophil adhesion by anti-αM or anti-β2 integrin antibody. Eosinophils from nonallergic donors were preincubated with mouse IgG₁, anti-αM integrin mAb, or anti-β2 integrin mAb (3 µg/mL each) for 15 minutes. Adhesion of the eosinophils to rh-periostin (1-10 µg/mL) was then assessed (n = 4). ****P* < .001 versus control. ###*P* < .001 versus mouse IgG₁. **B**, Effect of anti-integrin antibody concentrations on the suppression of eosinophil adhesion induced by periostin. Eosinophils from nonallergic donors were preincubated with mouse IgG₁, anti-αM integrin mAb, or anti-β2 integrin mAb (0.3-3 µg/mL) for 15 minutes. The adhesion of the eosinophils to rh-periostin (10 µg/mL) was then assessed (n = 4). ****P* < .001 versus mouse IgG₁.

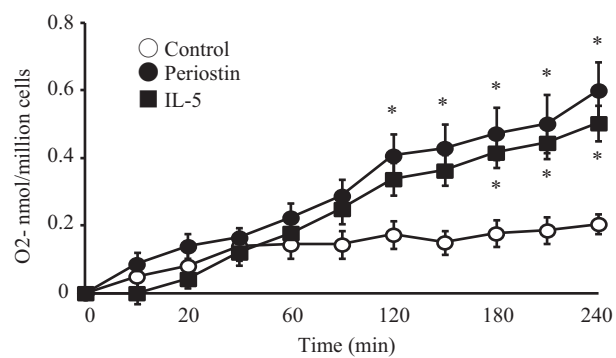


FIG E3. Effect of periostin on eosinophil O_2^- generation. Eosinophils (1×10^6 cells/mL) from nonallergic donors were incubated with or without IL-5 (100 pM) in periostin-coated plates (10 μ g/mL) or -uncoated plates. Eosinophil O_2^- generation was then examined ($n = 6$). Reaction kinetics over the next 240 minutes of eosinophil O_2^- generation are shown. * $P < .05$ versus control.

TABLE E1. Production of cytokines and chemokines by eosinophils stimulated with periostin

Cytokine (pg/mL)	Control	Periostin
IL-1 β	10.9 $\pm$ 5.4	74.2 $\pm$ 4.3*
IL-6	604.1 $\pm$ 299.4	2875.5 $\pm$ 276.0†
IL-8	3089.4 $\pm$ 1236.6	9383.0 $\pm$ 1535.3*
IFN- γ	36.0 $\pm$ 3.0	67.3 $\pm$ 5.7*
MCP-1	121.2 $\pm$ 56.2	360.8 $\pm$ 36.6‡
MIP-1 α	412.7 $\pm$ 72.7	762.9 $\pm$ 110.8‡
MIP-1 β	113.4 $\pm$ 32.6	1397.7 $\pm$ 229.1‡
TNF- α	92.6 $\pm$ 26.2	213.3 $\pm$ 38.2‡

Eosinophils from nonallergic donors in RPMI 1640 with 10% FBS were incubated with periostin for 24 hours, and the concentrations of cytokines and chemokines in cell-free supernatants were measured using Bio-Plex assay kits (n = 6).

MCP, Monocyte chemotactic protein; *MIP*, macrophage inflammatory protein.

* $P < .01$ versus cytokine/chemokine concentrations without periostin (control).

† $P < .001$ versus cytokine/chemokine concentrations without periostin (control).

‡ $P < .05$ versus cytokine/chemokine concentrations without periostin (control).