

Periostin: A novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals

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Background: Subepithelial fibrosis is a cardinal feature of bronchial asthma. Collagen I, III, and V; fibronectin; and tenascin-C are deposited in the lamina reticularis. Extensive evidence supports the pivotal role of IL-4 and IL-13 in subepithelial fibrosis; however, the precise mechanism remains unclear. We have previously identified the *POSTN* gene encoding periostin as an IL-4/IL-13-inducible gene in bronchial epithelial cells. Periostin is thought to be an adhesion molecule because it possesses 4 fasciclin I domains.

Objective: We explore the possibility that periostin is involved in subepithelial fibrosis in bronchial asthma.

Methods: We analyzed induction of periostin in lung fibroblasts by IL-4 or IL-13. We next analyzed expression of periostin in patients with asthma and in ovalbumin-sensitized and ovalbumin-inhaled mice. Furthermore, we examined the binding ability of periostin to other extracellular matrix proteins.

Results: Both IL-4 and IL-13 induced secretion of periostin in lung fibroblasts independently of TGF- β . Periostin colocalized with other extracellular matrix proteins involved in subepithelial fibrosis in both asthma patients and ovalbumin-sensitized and ovalbumin-inhaled wild-type mice, but not in either IL-4 or IL-13 knockout mice. Periostin had an ability to bind to fibronectin, tenascin-C, collagen V, and periostin itself. **Conclusion:** Periostin secreted by lung fibroblasts in response to IL-4 and/or IL-13 is a novel component of subepithelial fibrosis in bronchial asthma. Periostin may contribute to this process by binding to other extracellular matrix proteins.

Clinical implications: Periostin induced by IL-4/IL-13 shows promise in inhibiting subepithelial fibrosis in bronchial asthma. (J Allergy Clin Immunol 2006;118:98-104.)

Key words: Bronchial asthma, IL-4, IL-13, periostin, TGF- β , subepithelial fibrosis, basement membrane, extracellular matrix protein, fibronectin, tenascin-C

Subepithelial fibrosis is a cardinal feature of bronchial asthma. Some references describe that subepithelial fibrosis can be seen in early stages of bronchial asthma and resists steroid treatment, whereas it is absent in chronic obstructive pulmonary disease.¹⁻³ Although the clinical significance of subepithelial fibrosis in bronchial asthma is controversial, extensive evidence supports its correlation with the clinical and functional severity of bronchial asthma.⁴⁻⁶ Subepithelial fibrosis is a result of thickening of the lamina reticularis, in which extracellular matrix (ECM) proteins—such as collagens I, III, and V; fibronectin; and tenascin-C—are deposited beneath the basal lamina (the “true” basement membrane). Fibroblasts rather than epithelial cells would be responsible for this process.^{2,7,8} However, not all of its deposited components have been characterized, and it remains obscure how these components generate the reticular structure.

IL-4 and IL-13, T_H2 cytokines sharing receptors and signal pathways, have a critical role in causing phenotypic or functional changes of bronchial asthma such as airway hyperresponsiveness, eosinophil infiltration, and mucus overproduction in model mice.⁹⁻¹² In addition to these effects, analyses of model mice of allergic asthma have shown that IL-4 and IL-13 have a pivotal role also in subepithelial fibrosis.¹³⁻¹⁶ One report has proposed that this IL-13-induced ECM deposition would be caused by TGF- β , because the TGF- β level was high in the lungs of IL-13-expressing mice, and blockage of TGF- β reduced IL-13-induced collagen deposition.¹⁷ However, it is unclear whether subepithelial fibrosis by IL-4 and IL-13 is explained by the TGF- β -dependent pathway alone.

We have previously identified IL-4-inducible or IL-13-inducible genes in human bronchial epithelial cells (HBECS), in which the *POSTN* gene encoding periostin was included.¹⁸ Periostin is a secreted protein originally isolated from an osteoblast cell line, composed of the cysteine-rich domain in its N-terminal portion, 4 tandemly

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Abbreviations used

ECM: Extracellular matrix
FAS1: Fasciclin I
HBEC: Human bronchial epithelial cell

lined fasciclin I (FAS1) domains in the middle, and the alternative splicing domain in its C-terminal portion.¹⁹ Because the FAS1 domain is characteristic of a family of adhesion molecules,^{20–27} it has been assumed that periostin is an adhesion molecule. However, if this is so, the characteristics of periostin are not yet well understood, and its involvement in the pathogenesis of bronchial asthma has not been reported. In this article, we explore the possibility that periostin is involved in subepithelial fibrosis of bronchial asthma downstream of IL-4 or IL-13 signals.

METHODS

Plasmids

POSTN cDNA was prepared by RT-PCR using mRNA derived from HBECs, and then that was incorporated into either pET30a (Novagen, Madison, Wis), or pMT/Bip/V5-HisA (Invitrogen, Carlsbad, Calif). In pET30a-periostin, the His-tagged sequence was attached to the N terminus of the *POSTN* cDNA coding the mature periostin protein starting at the 22nd amino acid. In pMT/Bip/V5-HisA-periostin, the Bip signal and the V5 epitope/His-tagged sequences were attached to the N and C terminus of the *POSTN* cDNA, respectively. The C-terminal truncated type of periostin (periostin-ΔC: stopping at the 631st amino acid) was generated by the PCR method using mutation-incorporated oligonucleotides as the primers.

Cells

Drosophila S2 cells were maintained with Schneider *Drosophila* medium (GIBCO BRL, Rockville, Md) according to the manufacturer's procedure. For the induction of recombinant proteins, S2 cells were incubated with *Drosophila*-SFM (GIBCO BRL) supplemented with 500 μmol/L CuSO₄ and 2 mmol/L L-glutamine for 3 days. HBECs and BEAS-2B cells were cultured as previously described.¹⁸ MRC5, a normal embryonic lung fibroblast cell line (Riken BioResource Center, Tsukuba, Japan), and primary fibroblasts were cultured with Dulbecco modified Eagle medium (Invitrogen) supplemented with 10% FCS, 100 μg/mL streptomycin, and 100 U/mL penicillin G. Lung tissues were obtained during lung surgeries at the Saga Medical School Hospital, after written informed consent was obtained before inclusion in the study, and primary lung fibroblasts were expanded and used at passages 2 to 4.

For stimulation, cells were cultured with 10 ng/mL human IL-4, 50 ng/mL human IL-13 (Peprotech, Rocky Hill, NJ), or 10 ng/mL human TGF-β₁ (kindly provided by Dr A. Nakao) for the indicated period. In some experiments, 10 μg/mL neutralizing anti-TGF-β Ab (also provided by Dr A. Nakao) or 500 ng/mL brefeldin A (Sigma-Aldrich, St Louis, Mo) was used. Brefeldin A was incubated for 8 hours, after incubation of the cells with IL-4 for 24 hours.

Recombinant proteins

pET30a-periostin was transfected together with pKJE7 (TaKaRa Bio Inc, Ohtsu, Japan) to an *Escherichia coli*, BL21 (DE3; Novagen).

pMT/Bip/V5-HisA-periostin was transfected to *Drosophila* S2 cells together with pCoHygro (Invitrogen) by Effectene transfection reagent (Promega, Madison, Wis), and stable transfectants were selected with 400 μg/mL hygromycin (Sigma-Aldrich). The recombinant protein was purified by Ni-NTA Agarose (Qiagen, Hilden, Germany).

RT-PCR

Total RNA was extracted by ISOGEN (Nippongene, Tokyo, Japan). The RT reaction primed with random hexamer was performed by using GeneAmp RNA PCR Kit (Applied Biosystems Japan, Tokyo, Japan). The PCR reaction was performed with cDNA as a template using the primers below after an initial 5-minute denaturation at 96°C, followed by 30 cycles of 96°C for 1 minute, 52°C for 1 minute, and 72°C for 1 minute. The PCR primers used were 5'-CTGCCAAACAAGTTATTGAGCTGGC-3' and 5'-AATAATGTCCAGTCTCCAGGTTG-3' for periostin, and 5'-GAAGGTGAA GGTCCGAGT-3' and 5'-GAAGATGGTGTATGGGATTTC-3' for glyceraldehydephosphate dehydrogenase (GAPDH), respectively.

Western blotting

Antiperiostin serum was generated by purified His-tagged or V5 epitope/His-tagged periostin possessing all of 17 to 21 exons in the C-terminal portion, together with complete Freund adjuvant (Sigma-Aldrich) for immunization of New Zealand White rabbits.

The samples were applied to SDS-PAGE, and then electrophoretically transferred to polyvinylidene difluoride membranes (GE Healthcare Bio-Sciences, Piscataway, NY). The membranes were blotted by antiperiostin serum, antifibronectin Ab (TaKaRa), or anti-tenascin-C Ab (kindly provided by Dr M. Kusakabe). The proteins were visualized by ECL (GE Healthcare Bio-Sciences).

Specimens of human bronchial tissues

Bronchial tissues examined for immunostaining were autopsy specimens derived from 7 patients with asthma and 8 patients without asthma. The 7 patients with bronchial asthma were from 14 to 85 years old, had died from bronchial asthma or other causes, and had had bronchial asthma for 2 to 60 years (mean, 17.4 years). The patients with asthma were classified by the criteria of Global Initiative for Asthma 2002 as step 1 (3 patients), step 2 (2 patients), and step 4 (2 patients). Two patients at step 4 had received oral prednisolone (5 mg/d for 2 years) or inhaled β-agonist and theophylline. Five patients of step 1 and 2 had had almost no drugs, except 1 patient (step 2) received oral prednisolone (20 mg/d for 8 years).

Generation of chronic asthma model mice and their evaluation

Seven-week-old BALB/c mice (Japan SLC, Shizuoka, Japan), IL-4 gene knockout mice,²⁸ and IL-13 gene knockout mice (backcrossed to BALB/c more than 8 times)²⁹ were used. Experiments were undertaken following the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animals Science (1987).

Chronic asthma model mice were generated, as previously reported.³⁰ Either wild-type or IL-4 or IL-13 knockout mice were actively sensitized by intraperitoneal injections of 50 μg ovalbumin (Seikagaku Kogyo, Tokyo, Japan) with 1 mg alum on day 0 and 12. Starting on day 22, they were exposed to ovalbumin (1% in PBS) or saline by inhalation for 30 minutes every day for 3 consecutive weeks. Each condition was analyzed in 6 to 8 mice. Histologic examination was performed 24 hours after the final antigen challenge. The lung sections were stained with periodic acid-Schiff or Masson trichrome. Measurements of TGF-β₁ and hydroxyproline were performed as previously described.¹⁶

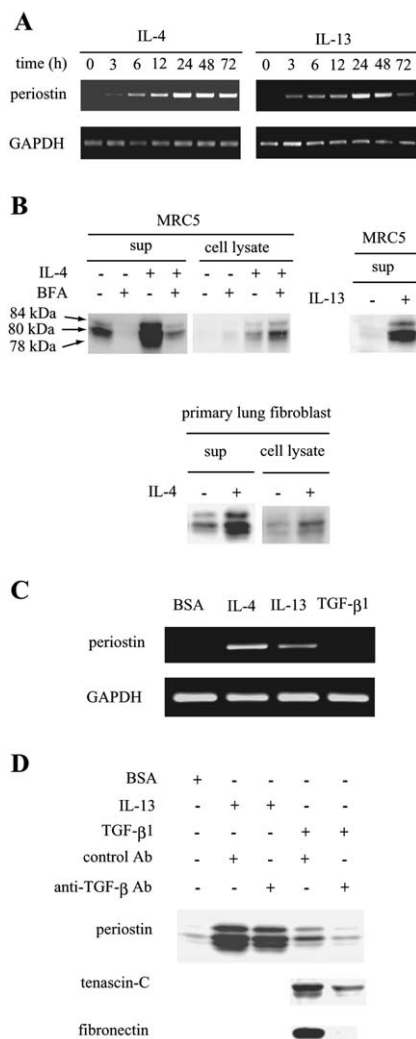


FIG 1. Induction of periostin by IL-4 and IL-13 in lung fibroblasts. Expression of periostin and GAPDH RNA in MRC5 cells (**A**, **C**) or periostin protein in supernatants (sup) or cell lysates of MRC5 cells or primary lung fibroblasts (**B**, **D**) stimulated with IL-4 or IL-13 or TGF- β 1 for the indicated times (**A**) or 72 hours (**B**, **D**) or 24 hours (**C**) was shown. Brefeldin A (BFA; **B**) or anti-TGF- β Ab or a control Ab (**D**) was present.

Immunohistochemical staining

After human or mouse lung tissues were fixed with 3.7% formaldehyde and processed by the standard methods, they were incubated with primary Ab or normal IgG overnight at 4°C. The antigen was detected by the ENVISION+/HRP (DAB) system (Dako Cytomation, Glostrup, Denmark). The primary Abs used for the experiments were antiperiostin serum, antifibronectin Ab, anti-tenascin-C Ab (Santa Cruz Biotechnology, Santa Cruz, Calif), and anticollagen I, III, V (DAIICHI FINE CHEMICAL, Takaoka, Japan), and IV (Dako Cytomation) Abs. Subepithelial fibrosis and deposition of periostin were examined by using a Leica image analysis system (Leica, Cambridge, United Kingdom), as previously described.¹⁶ Three Masson trichrome-stained or immuno-stained bronchioles of each specimen, in which the total length of the epithelial basement membrane was 3.0 to 9.0 μ m (human) or 1.0 to 2.5 μ m (mice), were selected, and the stained area including the basement membrane in 6 μ m (human) or beneath the basement membrane in 10 μ m (mice) was measured.

Binding assay

We coated the 96-well plates with 2.5 μ g/mL fibronectin; tenascin-C (CHEMICON International, Temecula, Calif); collagen I, III, or V (MORINAGA, Yokohama, Japan); BSA (Wako, Osaka, Japan); or periostin expressed in *Drosophila* S2 cells. After blocking with the blocking buffer (Tris-buffered saline containing 3% BSA, and 0.05% Tween-20), the indicated concentrations of biotinylated periostin, fibronectin, or BSA in the binding buffer (Tris-buffered saline containing 1% BSA, 0.05% Tween-20, and 10 mmol/L CaCl₂) were incubated overnight at 4°C. After extensive washing, streptavidin-conjugated horseradish peroxidase was incubated, and then the signals were developed by the ABTS Peroxidase Substrate System (KPL, Gaithersburg, Md). The data were corrected by evaluating the degree of biotinylation of each protein.

RESULTS

Induction of periostin by IL-4 or IL-13 in lung fibroblasts

By using both the microarray approach and quantitative RT-PCR analysis, we have previously demonstrated that the *POSTN* gene encoding periostin is an IL-4-inducible or IL-13-inducible gene in HBECs.¹⁸ To explore the possibility that periostin is induced by IL-4 or IL-13 also in lung fibroblasts, we first examined expression of periostin by IL-4 or IL-13 in a normal embryonic lung fibroblast cell line, MRC5, using RT-PCR. On stimulation of IL-4 or IL-13, expression of periostin became detectable at 3 hours and reached a peak after 24 hours (Fig 1, **A**). In accordance with expression of periostin at the mRNA level, either IL-4 or IL-13 caused expression of periostin at the protein level in both cell lysates and supernatants of MRC5 cells and primary lung fibroblasts (Fig 1, **B**, data not shown). The secreted periostin showed 3 sizes of mobilities on the gel—84, 80, and 78 kd, respectively—which were generated by alternative splicing, as described below. Brefeldin A significantly decreased secretion of periostin in the supernatants and accumulated the protein in the cell lysates (Fig 1, **B**). In contrast, either IL-4 or IL-13 induced expression of periostin in HBECs at the mRNA level, but not at the protein level (data not shown). These results demonstrated that IL-4 or IL-13 induces secretion of periostin in lung fibroblasts.

Because it is known that IL-13 induces expression of TGF- β , a fibrogenic cytokine in the lung,¹⁷ it would be possible that production of periostin by IL-4 or IL-13 is a result of TGF- β induced by IL-4 or IL-13. However, TGF- β 1 induced only low amounts of periostin compared with IL-4 or IL-13 (Fig 1, **C** and **D**). Furthermore, anti-TGF- β Ab blocked most secretion of tenascin-C and fibronectin induced by TGF- β , but blocked only slightly the secretion of periostin induced by either IL-4 or IL-13 (Fig 1, **D**, data not shown). These results demonstrated that expression of periostin by IL-4 or IL-13 in lung fibroblasts was independent of TGF- β .

There exist several isoforms of periostin, caused by alternative splicing in its C-terminal domain (accession numbers: AY140646, D13665, and D13666). To identify the isoform dominantly expressed in lung fibroblasts, we

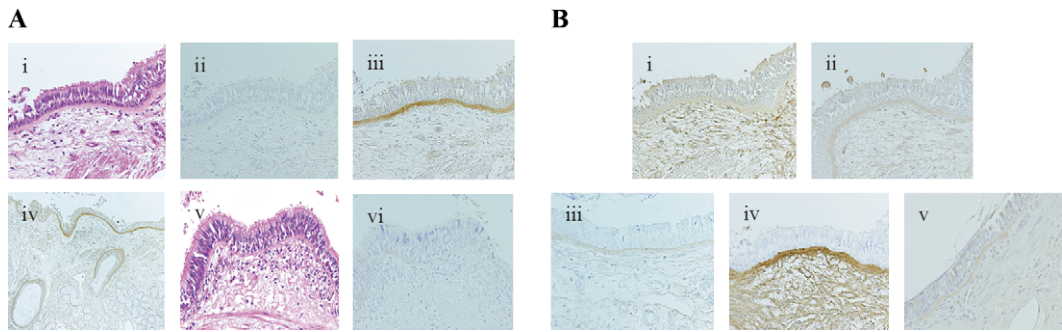


FIG 2. Immunohistochemical staining in bronchial tissues of patients with asthma. **A**, The bronchial tissues of patients with (*i-iv*) or without asthma (*v, vi*) were stained with hematoxylin & eosin (*i, v*) or incubated with preimmune (*ii*) or anti-periostin serum (*iii, iv, vi*). Panel *iv* shows the low-magnitude view of panel *iii*. **B**, The bronchial tissues of patients with asthma were incubated with antifibronectin Ab (*i*), anti-tenascin-C Ab (*ii*), or anticollagen I (*iii*), III (*iv*), or V (*v*) Abs.

TABLE I. Deposition of Periostin in patients with asthma and ovalbumin-sensitized and ovalbumin-inhaled mice

Human	n	Age (y)	Fibrotic area (area/length of BM)	Periostin-stained area (area/length of BM)
Nonasthma	8	63.6 ± 13.8	1.27 ± 0.24	1.02 ± 0.39
Asthma	7	58.1 ± 22.3	2.97 ± 0.84*	3.15 ± 0.45†

Mouse	n	TGF-β ₁ (pg/mL)	Hydroxyproline (μg/lung)	fibrotic area (area/length of BM)	Periostin-stained area (area/length of BM)
WT-saline	7	29.3 ± 4.8	129.4 ± 3.0	1.07 ± 0.05	4.61 ± 0.72
IL-4 KO-saline	6	35.0 ± 7.4	131.3 ± 3.8	1.01 ± 0.06	3.70 ± 0.42
IL-13 KO-saline	6	53.7 ± 8.3	138.8 ± 2.2	1.12 ± 0.10	3.20 ± 1.15
WT-OVA	8	626.3 ± 69.4‡	178.4 ± 7.9§	2.52 ± 0.13§	6.43 ± 0.78§
IL-4 KO-OVA	8	105.9 ± 11.3 *	137.1 ± 6.1¶	1.26 ± 0.10 #	3.52 ± 0.59
IL-13 KO-OVA	8	125.2 ± 11.4 **	138.7 ± 2.5	1.24 ± 0.06	3.98 ± 0.63

BM, Basement membrane, KO, knockout; WT, wild-type.
P* < .005; †*P* < .001 (vs nonasthma); ‡*P* < .01; §*P* < .001 (vs WT-saline); ||*P* < .001; ¶*P* < .01 (vs WT-OVA); #*P* < .05; *P* < .001 (vs KO-saline).

sequenced the transcripts of periostin induced by IL-4 in MRC5 cells (data not shown). More than half of the investigated transcript was the type containing exons 19 and 20 and lacking exons 17, 18, and 21 in its C-terminal domain. The second and third dominant transcripts were the types expressing exon 20 alone, and exons 18, 19, and 20, respectively. Three sizes of mobilities of the secreted periostin on the gel—84, 80, and 78 kd, respectively—would correspond to these 3 major transcripts. On the basis of these results, we denoted the most dominantly expressed type of periostin in MRC5 cells as periostin-L (the lung type of periostin).

Deposition of periostin in bronchial tissues of patients with asthma

We next analyzed distribution of periostin in bronchial tissues of patients with and without asthma. Subepithelial portions, observed by light microscopy to be homogeneous and hyaline, were obviously thickened in patients with bronchial asthma, with periostin specifically localized, whereas deposition of periostin was not detected in bronchial tissues of patients without asthma (Fig

2, A). Deposition of periostin in subepithelial portions was observed in all bronchial tissues derived from the 7 investigated patients, regardless of their various backgrounds (Table I). Periostin colocalized with other ECM proteins such as collagens I, III, and V; fibronectin; and tenascin-C, deposited in subepithelial portions of asthma patients, as previously described (Fig 2, B).^{31,32} These results demonstrated that periostin was a novel matrix protein involved in subepithelial fibrosis of patients with bronchial asthma.

Deposition of periostin in bronchial tissues of ovalbumin-sensitized and ovalbumin-inhaled mice

Considering our findings that periostin was induced by IL-4 or IL-13 in lung fibroblasts and that periostin was a novel component of subepithelial fibrosis in patients with bronchial asthma, we reasoned that IL-4 and/or IL-13 would be involved in subepithelial fibrosis by secretion of periostin. To explore this possibility, we generated a chronic asthma model by using wild-type mice or IL-4 or IL-13 knockout mice and analyzed deposition of periostin. In this

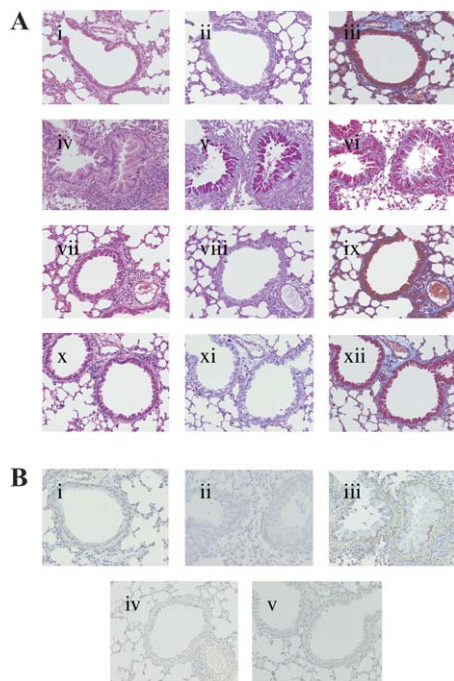


FIG 3. Immunohistochemical staining in bronchial tissues of chronic asthma model mice. **A**, The bronchial tissues of saline-inhaled (*i-iii*) or ovalbumin-inhaled (*iv-vi*) wild-type mice or ovalbumin-inhaled IL-4 (*vii-ix*) or IL-13 (*x-xii*) knockout mice were stained with hematoxylin & eosin (*i, iv, vii, x*) or periodic acid-Schiff (*ii, v, viii, xi*) or Masson trichrome (*iii, vi, ix, xii*). **B**, The bronchial tissues of saline-inhaled (*i*) or ovalbumin-inhaled (*ii, iii*) wild-type mice or ovalbumin-inhaled IL-4 (*iv*) or IL-13 (*v*) knockout mice were incubated with preimmune (*ii*) or antiperiostin serum (*i, iii-v*).

chronic asthma model, subepithelial fibrosis appeared in ovalbumin-inhaled wild-type mice, but not in saline-inhaled mice, and was significantly decreased in ovalbumin-inhaled IL-4 or IL-13 knockout mice, as well as goblet cell hyperplasia, infiltration of inflammatory cells, and production of TGF- β_1 and hydroxyproline, as described in previous reports (Fig 3, A; Table I).¹⁴⁻¹⁶ In parallel with subepithelial fibrosis, periostin was also deposited in subepithelial portions in ovalbumin-inhaled wild-type mice, but not in saline-inhaled mice, and was significantly decreased in IL-4 or IL-13 knockout mice (Fig 3, B, and Table I). These results suggested that periostin was involved in subepithelial fibrosis of bronchial asthma downstream of IL-4 and/or IL-13 signals.

Binding of periostin with ECM proteins in heterophilic and homophilic manners

Our findings that periostin colocalized with fibronectin, tenascin-C, and collagens, known components of subepithelial fibrosis of bronchial asthma, indicated that periostin forms a reticular structure by binding to these ECM proteins. Because periostin has a tandem repeat of 4 FAS1 domains in the middle, which are thought to be important for its adhesive activity,²⁰⁻²⁷ we analyzed the binding abilities of periostin-L and mutated periostin, truncating

its C-terminal portion but keeping 4 FAS1 domains (periostin- Δ C) to these ECM proteins (Fig 4, A). Periostin-L bound to fibronectin, tenascin-C, and collagen V in a dose-dependent manner, but not to collagens I and III, and periostin- Δ C showed much stronger binding to these ECM proteins than periostin-L (Fig 4, B). These results indicate that the FAS1 domains would be primary binding sites to these ECM proteins, and that the C-terminal portion of periostin-L may downregulate its binding ability to these ECM proteins.

We next analyzed binding ability of periostin to periostin itself. It turned out that periostin did bind to itself, and that the binding ability of periostin- Δ C to either periostin-L or periostin- Δ C was higher than that of periostin-L (Fig 4, C). These results indicated that the FAS1 domains of periostin could bind to each other, in addition to other ECM proteins. Taking these results together, periostin appears to have the ability to bind to ECM proteins such as tenascin-C, fibronectin, collagen V, and periostin itself, in both heterophilic and homophilic manners, which may explain how periostin would contribute to subepithelial fibrosis in bronchial asthma.

DISCUSSION

Subepithelial fibrosis is a cardinal feature of bronchial asthma. It is known that deposition of collagens I, III, and V; fibronectin; and tenascin-C in the basal lamina contributes to this process.^{7,8} Our current study shows that periostin secreted from lung fibroblasts is a previously unrecognized component of subepithelial fibrosis (Figs 1-3; Table I). Periostin was originally identified as a gene expressed in a mouse osteoblastic cell line¹⁹; however, its pathophysiological roles remain poorly understood. It has been suggested that periostin plays a role in development of bone, tooth, heart, and myoseptum.³³⁻³⁵ There are also reports that periostin is involved in the pathogenesis of heart failure and carcinogenesis.^{36,37} However, there is no report demonstrating pathophysiological roles of periostin in bronchial tissues or bronchial asthma, which has been highlighted by our current findings.

It is widely known that IL-4 and IL-13 are involved in subepithelial fibrosis of bronchial asthma.¹³⁻¹⁶ Deposition of ECM proteins by IL-13 is assumed to be dependent on TGF- β .¹⁷ However, our current findings—that secretion of periostin from lung fibroblasts is dependent on IL-4 and IL-13, but not on TGF- β (Fig 1, C and D)—indicated the possibility that there exists a TGF- β -independent pathway in addition to the TGF- β -dependent pathway. We have described how IL-4 or IL-13 induced expression of periostin, but not tenascin-C or fibronectin, whereas TGF- β dominantly induced expression of tenascin-C and fibronectin (Fig 1, C and D), and furthermore that periostin bound to tenascin-C, fibronectin, and collagen V (Fig 4, A). Together, these results suggest that IL-4/IL-13 and TGF- β would cooperate with each other, secreting the components required for the subepithelial fibrosis. In ovalbumin-inhaled IL-4 or IL-13 knockout mice, expression of both TGF- β and

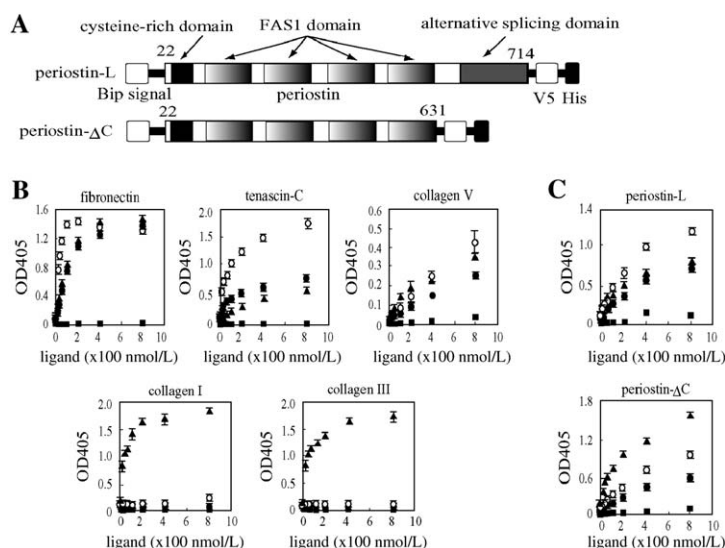


FIG 4. Binding assay of periostin to other ECM proteins or periostin. **A**, A schematic model of the structure of recombinant proteins is depicted. The numbers represent the amino acids starting or stopping the encoded region of periostin. **B**, **C**, Binding of the indicated concentrations of biotinylated periostin-L (closed circle), periostin-ΔC (open circle), fibronectin (closed triangle), or BSA (closed square) to tenascin-C; fibronectin; or collagen I, III, or V (**B**) or periostin-L or periostin-ΔC (**C**).

periostin was decreased compared with ovalbumin-inhaled wild-type mice (Table I). Downregulation of periostin expression would be independent of, not caused by, decrease of TGF- β , because of a weak ability of TGF- β to induce periostin. It would be of interest to analyze the effects of blocking TGF- β in ovalbumin-inhaled mice or administration of TGF- β to naive mice on expression of periostin.

Our current study showed that periostin bound to fibronectin, tenascin-C, collagen V, and periostin itself, but not to collagens I and III, and that the FAS1 domains in periostin would be responsible for these bindings (Fig 4). The FAS1 domain was originally identified in a *Drosophila* membrane-anchored protein, fasciclin I, which plays a critical role in axon guidance.^{20,21} FAS1 domain exists in many proteins derived from various species including bacteria²² and plants,²³ which suggests that this domain represents an evolutionarily ancient adhesion domain. In human beings, FEEL/stabilin-1/2, TGF- β -inducible gene-h3, and periostin have been described as possessing the FAS1 domain.^{19,25-27} FEEL/stabilin-1/2 are known to act as scavenger and hyaluronan receptors. TGF- β -inducible gene-h3, a highly homologous matrix protein with periostin, binds to ECM proteins and integrins.³⁸⁻⁴⁰ Our current study is the first to show binding of periostin to ECM proteins in both heterophilic and homophilic manners.

In conclusion, periostin is a novel component involved in subepithelial fibrosis in bronchial asthma downstream of IL-4 and IL-13 signals. These results expand our understanding of the pathogenesis of subepithelial fibrosis in bronchial asthma. Furthermore, our current findings would support the notion that IL-4/IL-13 signals would be also a promising target to inhibit subepithelial fibrosis in bronchial asthma.

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