

R&D Systems Quality-Tested Antibodies

Over 10,000 antibodies for a wide variety of applications including:

✓ Western Blot ✓ Flow Cytometry ✓ Immunocytochemistry ✓ Neutralization

[Click here for more information](#)



This information is current as of March 15, 2011

Periostin Regulates Goblet Cell Metaplasia in a Model of Allergic Airway Inflammation

Sarita Sehra, Weiguo Yao, Evelyn T. Nguyen, Ayele-Nati N. Ahyi, Florencia M. Barbé Tuana, Shawn K. Ahlfeld, Paige Snider, Robert S. Tepper, Irina Petrache, Simon J. Conway and Mark H. Kaplan

J Immunol; Prepublished online 14 March 2011;

doi:10.4049/jimmunol.1002359

<http://www.jimmunol.org/content/early/2011/03/14/jimmunol.1002359>

Subscriptions	Information about subscribing to <i>The Journal of Immunology</i> is online at http://www.jimmunol.org/subscriptions
Permissions	Submit copyright permission requests at http://www.aai.org/ji/copyright.html
Email Alerts	Receive free email-alerts when new articles cite this article. Sign up at http://www.jimmunol.org/etoc/subscriptions.shtml/

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.
Copyright ©2011 by The American Association of
Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



Periostin Regulates Goblet Cell Metaplasia in a Model of Allergic Airway Inflammation

Sarita Sehra,* Weiguo Yao,* Evelyn T. Nguyen,* Ayele-Nati N. Ahyi,*[†]
 Florencia M. Barbé Tuana,* Shawn K. Ahlfeld,* Paige Snider,* Robert S. Tepper,*
 Irina Petrache,[‡] Simon J. Conway,* and Mark H. Kaplan*[†]

Periostin is a 90-kDa member of the fasciclin-containing family and functions as part of the extracellular matrix. Periostin is expressed in a variety of tissues and expression is increased in airway epithelial cells from asthmatic patients. Recent studies have implicated a role for periostin in allergic eosinophilic esophagitis. To further define a role for periostin in Th2-mediated inflammatory diseases such as asthma, we studied the development of allergic pulmonary inflammation in periostin-deficient mice. Sensitization and challenge of periostin-deficient mice with OVA resulted in increased peripheral Th2 responses compared with control mice. In the lungs, periostin deficiency resulted in increased airway resistance and significantly enhanced mucus production by goblet cells concomitant with increased expression of *Gob5* and *Muc5ac* compared with wild type littermates. Periostin also inhibited the expression of *Gob5*, a putative calcium-activated chloride channel involved in the regulation of mucus production, in primary murine airway epithelial cells. Our studies suggest that periostin may be part of a negative-feedback loop regulating allergic inflammation that could be therapeutic in the treatment of atopic disease. *The Journal of Immunology*, 2011, 186: 000–000.

Allergic asthma is a Th2-mediated inflammatory disease characterized by pulmonary eosinophilia, airway hyperresponsiveness (AHR) and mucus overproduction by goblet cells (1, 2). With subsequent progression of this disease, airways undergo structural and phenotypic changes resulting in airway remodeling (3) including damage to epithelial cells, goblet cell metaplasia (GCM), subepithelial fibrosis, and smooth muscle hyperplasia and hypertrophy (4). One of the key features of airway remodeling is the development of GCM, which leads to airflow obstruction in asthma. Mortality of asthma patients reveal mucus plugging on postmortem examination. Several studies have implicated that the Th2-cytokines IL-4/IL-9/IL-13 play critical roles in inducing mucus production in asthma (5–9). However, the mechanism underlying how Th2 cytokines induce mucus production is poorly understood. Mouse calcium-activated chloride channel-3 (mCLCA-3; gob-5)/human CLCA-1, acting as a downstream molecule of the Th2 cytokines IL-4/IL-9/IL-13, has been suggested to be important in mucus production (10). Regulatory

pathways controlling mucus production need to be explored further to alleviate excess mucus production in respiratory diseases including asthma.

Periostin, an extracellular matrix protein originally isolated from an osteoblast cell line, is induced by IL-4 and IL-13 in airway epithelial cells (11, 12). Periostin is secreted by fibroblasts and composed of a cysteine-rich domain in its N-terminal end, four tandem fasciclin I (FASI) domains, and an alternatively spliced domain at the C-terminal end (13). Periostin shares structural homology to FASI identified in insects, and other mammalian proteins including β ig-h3, stabilin I and II, MBP-70, Algal-CAM, and Periostin-like-factor (13–19). IL-13 and TGF- β induce the expression of periostin in esophageal fibroblasts, and there is increased periostin expression in eosinophilic esophagitis (EE) biopsy samples (20). Periostin-deficient (*Postn*^{−/−}) mice subjected to intranasal *Aspergillus* infection had decreased eosinophilic inflammation in the lungs and esophagus (20).

In this report, we examine the development of allergen-induced pulmonary inflammation, examining the effects of periostin deficiency on peripheral Th2 responses, lung function and mucus production in mice. Our study demonstrates an important role for periostin in the regulation of mucus production, as deficiency of periostin significantly increased mucus expression by goblet cells and augmented airway resistance. However, periostin deficiency did not affect allergic airway inflammation, indicating that periostin function in allergic airway disease is restricted to a subset of features including GCM.

Materials and Methods

Mice

Postn^{−/−} mice on a mixed genetic background (B6/129) were generated as described previously (21). Briefly, the *Postn* gene was targeted in embryonic stem cells, by replacing exons 4–10 encoding three of the four fasciclin domains, which produced a null allele. Control mice were wild type (WT) littermates. All mice were maintained in specific pathogen-free conditions and experiments were approved by the Indiana University Institutional Animal Care and Use Committee.

*Department of Pediatrics, Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202; [†]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46202; and [‡]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

Received for publication July 13, 2010. Accepted for publication February 13, 2011.

This work was supported in part by the Riley Children's Foundation (to S.J.C. and P.S.) and the Indiana University Department of Pediatrics (Cardiology) and Public Health Service Awards U19 AI070448 (to M.H.K. and R.S.T.), R01 HL080071 (to R.S.T.), and R01 HL092508 (to S.J.C.).

Address correspondence and reprint requests to Dr. Mark H. Kaplan and Dr. Sarita Sehra, Department of Pediatrics, Indiana University School of Medicine, Herman B. Wells Center for Pediatric Research, 1044 West Walnut Street, Indianapolis, IN 46202. E-mail addresses: mkaplan2@iupui.edu and sasehra@iupui.edu

Abbreviations used in this article: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; DC, dendritic cell; EE, eosinophilic esophagitis; FASI, fasciclin I; GCM, goblet cell metaplasia; mCLCA-3, mouse calcium-activated chloride channel-3; MTEC, mouse tracheal epithelial cells; NS, nonsensitized; PAS, periodic acid-Schiff; Penh, enhanced pause; WT, wild type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/\$16.00

Flow cytometry

Splenocytes or bronchoalveolar lavage (BAL) cells ($0.5\text{--}1.0 \times 10^6$ per sample) were washed and stained in PBS with 2% BSA and 0.1% NaN₃ (FACS buffer). Cells were first incubated with anti-FcR Abs, clone 2.4G2 (BD Pharmingen), for 10 min. All staining was done at 4°C, followed by one wash with FACS buffer, then fixed in PBS with 2% BSA, 0.1% NaN₃, and 0.5% formaldehyde. Samples were stained with Abs directly conju-

gated to FITC, PE, CyChrome, or allophycocyanin (BD Pharmingen) for CD4, CD8, CD3, B220, CD44, CD62L, CD11c, CD3, B220, CCR3, and MHC class II (BD Pharmingen). Analysis of lymphocyte populations was performed using a forward scatter (size) gate, and the expression of CD44 and CD62L were determined on CD4⁺ cells. The cellular composition of BAL for the populations of eosinophils, neutrophils, T cells, B cells, dendritic cells (DCs), and macrophages was performed as described by a flow cytometric method (22). Eosinophils, neutrophils, T cells, B cells, and mononuclear cells were distinguished by cell size and the expression of CD3, B220, CCR3, CD11c, and MHC class II.

Analysis of Th cell differentiation

Total CD4⁺ T cells were isolated from spleens of *Postn*^{-/-} mice and WT littermates on a mixed background (MACS isolation system; Miltenyi Biotec, Auburn, CA). T cells were activated with plate-bound anti-CD3 (4

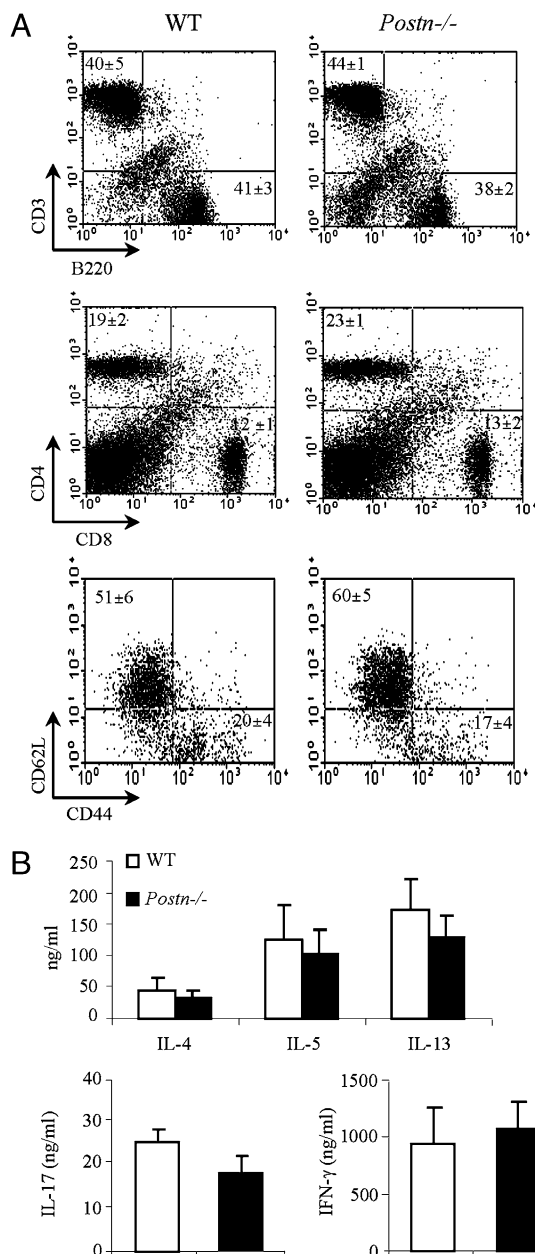


FIGURE 1. Immune cell profile and cytokine levels in WT and *Postn*^{-/-} mice. **A**, Splenocytes from WT and *Postn*^{-/-} mice were analyzed by FACS, by staining with the Abs for B220 and CD3, CD4 and CD8, and CD44 and CD62L. Percentages of CD3⁺, CD4⁺, CD8⁺, B220⁺, CD62L⁺, and CD44⁺ cells in the respective quadrants are indicated (mean ± SD). Cells were gated for lymphocyte size by forward scatter. CD44 and CD62L expression was examined by gating on CD4⁺ cells. **B**, CD4⁺ T cells were cultured under Th2 (IL-4 + anti-IFN-γ), Th17 (TGF-β + IL-6 + IL-1β along with blocking Abs to IFN-γ and IL-4), or Th1 (IL-12 + anti-IL-4), priming conditions for 5 d. Cells were restimulated, and 24-h supernatants were analyzed for cytokine content by ELISA. The levels of IL-4, IL-5, IL-13, IL-17, and IFN-γ from cultured CD4⁺ T cells under Th2, Th17 and Th1 conditions respectively are shown. Data are expressed as mean ± SE of three to four WT or *Postn*^{-/-} mice and representative of three experiments.

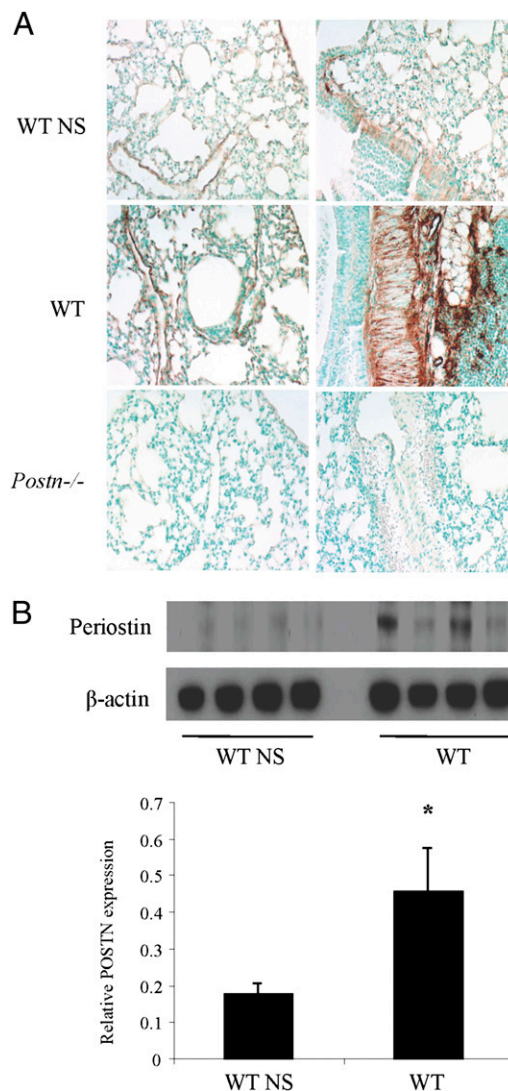


FIGURE 2. Induction of periostin in lungs of WT mice. **A**, Immunohistochemistry was performed on paraffin-embedded lung tissue from WT NS mice (top panel), sensitized, and challenged WT mice (middle panel) or *Postn*^{-/-} mice (*Postn*^{-/-}, bottom panel). Original magnification ×100. Periostin ECM deposition is predominantly observed within the submucosa and lung parenchyma surrounding the bronchus and bronchioles. Specificity of the periostin Ab and protein induction was verified via use of *Postn*^{-/-} sensitized and challenged mice. Similar results were observed with four separate mice of each genotype and treatment. **B**, Immunoblots of protein extracts from lung sections of NS and sensitized and challenged mice for periostin with β-actin as the loading control. Bar graphs represent the average densitometry of four samples normalized to expression of β-actin. *Significantly different from NS ($p < 0.05$).

$\mu\text{g/ml}$ 145-2C11) and soluble anti-CD28 (1 $\mu\text{g/ml}$; BD Pharmingen) and cultured under conditions that prime Th1 (IL-12 at 10 ng/ml and anti-IL-4 at 10 $\mu\text{g/ml}$), Th2 (IL-4 at 10 ng/ml [PeproTech, Rocky Hill, NJ] and anti-IFN- γ at 10 $\mu\text{g/ml}$), and Th17 (TGF- β 1 [5 ng/ml] and IL-6 [100 ng/ml; PeproTech] + IL-1 β [10 ng/ml] + anti-IL-4 [10 $\mu\text{g/ml}$] + anti-IFN- γ [10 $\mu\text{g/ml}$]) conditions. After 5 d in culture, cells were restimulated with plate-bound anti-CD3 (4 $\mu\text{g/ml}$) for 24 h, and cell-free supernatants were analyzed for IFN- γ , IL-4, and IL-17 using ELISA (reagents from BD Pharmingen) (23).

Induction of allergic airway inflammation

Mice were sensitized by i.p. injections of OVA adsorbed with alum (OVA and alum from Sigma-Aldrich, St. Louis, MO) at a dose of 20 μg OVA/2 mg alum on days 0 and 7. On day 14, mice were exposed to intranasal OVA (100 μg) per day for 6 consecutive days. Mice were euthanized by i.p. injection of pentobarbital (5 mg/mouse), 48 h after the last intranasal challenge. The trachea was cannulated and lungs lavaged three times with 1 ml PBS. The cells recovered in BAL fluid were counted with a hemocytometer. Eosinophils, neutrophils, T cells, B cells, and mononuclear cells in the BAL were distinguished by cell size and the expression of CD3, B220, CCR3, CD11c, and MHC class II, as described by a flow cytometric method (22). Cytokine levels in cell-free BAL fluid were measured using ELISA. Total BAL cells were stimulated with 4 $\mu\text{g/ml}$ anti-CD3 or 100 $\mu\text{g/ml}$ OVA for 72 h before supernatants were tested for cytokine levels using ELISA. For quantitative PCR measurements, lung tissues were homogenized in a tissue lyser (Qiagen), and RNA isolated with RNeasy kit (Qiagen) was used to synthesize cDNA for subsequent analysis. Peripheral immune responses in sensitized and challenged WT or *Postn*^{-/-} mice were assessed by culturing splenocytes with anti-CD3 (4 $\mu\text{g/ml}$) or OVA (100 $\mu\text{g/ml}$) for 72 h before cell-free supernatants were tested for cytokines using ELISA.

AHR and airway inflammation

AHR to methacholine challenge (dose range from 10 to 200 mg/ml) was determined 24 h after the last intranasal challenge. Noninvasive unrestrained whole-body plethysmography (Buxco Systems, Wilmington, NC) was used to record airway responsiveness via a dimensionless parameter termed enhanced pause (Penh) to estimate the total pulmonary

resistance, an indicator of bronchoconstriction. In separate animals, AHR was measured by invasive means of determining airway resistance (Buxco Systems). Mice were anesthetized and trachea intubated, and mice were ventilated before intratracheally delivering nebulized saline, followed by methacholine doses of 5–200 mg/ml.

Histological and immunohistochemical analysis

Lung tissue derived from mice 48 h after the last intranasal challenge was fixed in 10% formalin and paraffin-embedded sections were stained with H&E or periodic acid-Schiff (PAS) to evaluate the infiltration of inflammatory cells and mucus secretion. Peribronchial and perivascular inflammation was assessed using light microscopy with $\times 400$ magnification. Mucus production in histological sections was assessed by measuring color intensity of PAS staining of goblet cells on histological sections, image capture with $\times 10$ magnification, in a blinded fashion, and quantitative intensity (expression) measurement of PAS staining exclusively in the large airways, using a macro in the Metamorph Imaging software (Universal), as described previously (24). Immunostaining of periostin (1/10,000 dilution) using the avidin–biotin complex kit (Vector Laboratories) following manufacturer's directions, was performed as described previously (21). Negative controls that lacked either the primary or secondary or both Abs, or *Postn*^{-/-} mice, did not produce any diaminobenzidine staining.

Western blot analysis of periostin expression in lung tissues

Lung tissue sections from nonsensitized (NS) and sensitized and challenged WT mice were snap frozen in liquid nitrogen. Tissues were homogenized as described previously (25). Western blot analysis was performed as previously described (26) using specific Abs against periostin or control Ab β -actin (Santa Cruz Biotechnology, Santa Cruz, CA). For densitometric analysis, the scanned images of immunoblots were quantified with Image J software.

Effect of periostin on Th2-differentiated cells and LPS-stimulated DCs

Periostin-containing and control supernatants were collected from HEK 293 cells transfected with full-length murine periostin cDNA (P. Snider and S. J. Conway, unpublished observations) or empty vector. Briefly, the CD4⁺

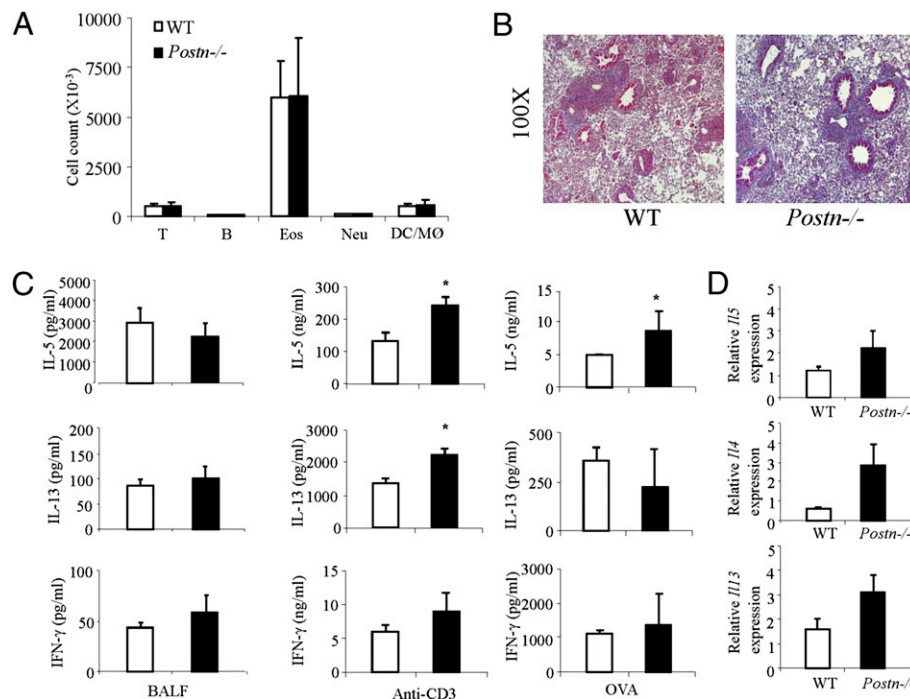


FIGURE 3. Effect of periostin-deficiency on BAL cell populations and cytokine secretion. **A**, BAL was performed in sensitized and challenged mice 48 h after the last challenge. Cell populations in BAL were stained with the respective Abs to identify eosinophils, neutrophils, T cells, B cells, and mononuclear cells. Results are expressed as mean $\pm$ SE from three mice and are representative of three experiments. **B**, Lungs of sensitized and challenged mice were embedded in paraffin and analyzed by H&E staining (original magnification $\times 100$). **C**, Cytokines IL-5, IL-13, and IFN- γ were determined in BAL fluid of sensitized and challenged mice by ELISA. BAL cells were stimulated with anti-CD3 (4 $\mu\text{g/ml}$) or OVA (100 $\mu\text{g/ml}$) for 72 h. Cell-free supernatants were analyzed for cytokines IL-5, IL-13, and IFN- γ by ELISA. Results are expressed as mean $\pm$ SE from three mice and are representative of three experiments. * $p < 0.05$ using a two-tailed Student *t* test, significantly different from WT. **D**, Total lung RNA was isolated from sensitized and challenged WT and *Postn*^{-/-} mice and analyzed for IL-4, IL-5, and IL-13 expression by quantitative PCR.

T cells from the spleens and lymph nodes of WT mice were cultured under Th2-priming conditions in the presence of periostin-containing or control media (10% v/v). After 5 d in culture, cells were restimulated with plate-bound anti-CD3 (4 μ g/ml) for 24 h and cell-free supernatants were analyzed for IL-4, IL-5, and IL-13 using ELISA (reagents from BD Pharmingen). The effect of periostin-containing or control media was also tested on CD11c⁺ DCs. Splenic DCs were isolated by positive selection with anti-CD11c magnetic beads following the manufacturer's instructions (Miltenyi Biotec). Isolated CD11c⁺ DCs were stimulated with LPS (1 μ g/ml) in the presence of periostin-containing or control media, or recombinant periostin (R&D Systems) for 72 h. Cell-free culture supernatants were tested for IFN- γ by ELISA. Next, we also stimulated CD11c⁺ DC with LPS (10 ng/ml) in the presence of recombinant periostin (0–100 ng/ml) and determined IFN- γ levels in 72 h culture supernatants by ELISA.

Mouse tracheal epithelial cell culture

Mouse tracheal epithelial cells (MTEC) from C57BL/6 mice were cultured as described previously (27). Briefly, tracheas were resected, opened longitudinally, incubated in Ham's F-12 pen-strep containing pronase followed by gentle inversion three times, to release the cells. Cells were then incubated with pancreatic DNase I and finally resuspended in MTEC basic media with 10% FBS. Fibroblasts were removed by incubation of the cells in tissue culture plates for 3–4 h at 37°C. Nonadherent cells were cultured for 48 h in 24-well plates. Once the cells reached confluence, cells were treated with varying concentrations of recombinant mouse IL-13 (R&D Systems, Minneapolis, MN) for 6 h to analyze gene expression. To test the effect of periostin on GCM-associated gene expression, MTEC were stimulated with varying concentrations of periostin for 6 h in the presence of 1 ng/ml IL-13. After 6 h, mucus gene expression was analyzed by TaqMan real-time quantitative PCR.

Results

Deficiency of periostin does not alter immune cell profile or cytokine secretion in vitro

Although *Postn*^{-/-} mice exhibit teeth, bone, and cardiac defects, and although they can be decreased in size, mice develop normally on wet feed (21). *Postn*^{-/-} mice used for our studies were comparable in size to their WT littermates. To determine whether *Postn*^{-/-} mice have altered lymphocyte development, splenocytes were analyzed for surface expression of CD3, B220, CD4, CD8, CD44, and CD62L by flow cytometry. B and T cell populations as well as populations of memory and naive CD4⁺ T cells remain unaltered in *Postn*^{-/-} mice (Fig. 1A).

To determine whether periostin deficiency had any effect on the ability of naive T cells to respond to cytokines and differentiate into effector subsets, purified CD4⁺ T cells from splenocytes were stimulated with anti-CD3 and anti-CD28 under Th1, Th2, and Th17 conditions. Analysis of cytokine production in cell-free culture supernatants revealed that *Postn*^{-/-} T cells had comparable levels of IFN- γ , IL-4, IL-5, IL-13, and IL-17 to WT littermates under Th1, Th2, and Th17 conditions, respectively (Fig. 1B). Thus, *Postn*^{-/-} mice have normal lymphocyte development in the periphery and T cells are unimpaired in their ability to acquire Th effector phenotypes.

Allergic airway inflammation in periostin-deficient mice

We next determined the role of periostin in allergic airway inflammation by using an OVA-induced model of sensitization and intranasal challenge. *Postn*^{-/-} mice and WT littermates were subjected to sensitization and challenge with OVA. To determine whether periostin is induced in the lungs of sensitized mice, we performed immunohistochemistry of paraffin-embedded tissue sections. Immunohistochemistry revealed that periostin is normally present at low levels within control WT NS lung sections, and staining is significantly increased in lung tissue from sensitized and challenged WT mice (Fig. 2A, middle panel). Periostin extracellular matrix deposition is predominantly observed within

the submucosa and lung parenchyma surrounding the bronchus and bronchioles. Staining was absent in sections from *Postn*^{-/-} sensitized and challenged mice (Fig. 2A, bottom panel).

Western blot analysis was used to confirm the induction of periostin expression. We observed a significant increase in periostin expression in the lung protein extracts from sensitized and challenged mice as compared with NS mice. Expression assessed by immunoblot was averaged from four mice and showed a 2.5-fold increase in the amount of periostin expression (Fig. 2B).

To determine whether periostin-deficiency affected pulmonary inflammation following sensitization and challenge, we next analyzed cells in the BAL for populations of T cells, B cells, eosinophils, neutrophils and dendritic cells by flow cytometry. Deficiency of periostin did not result in significant differences in the predominant T cell and eosinophil populations augmented in allergic airway inflammation over NS mice that did not show any inflammation (Fig. 3A). Histopathological analysis of lung sections did not reveal differences in the amount of inflammation in the lungs of *Postn*^{-/-} mice compared with WT littermates (Fig.

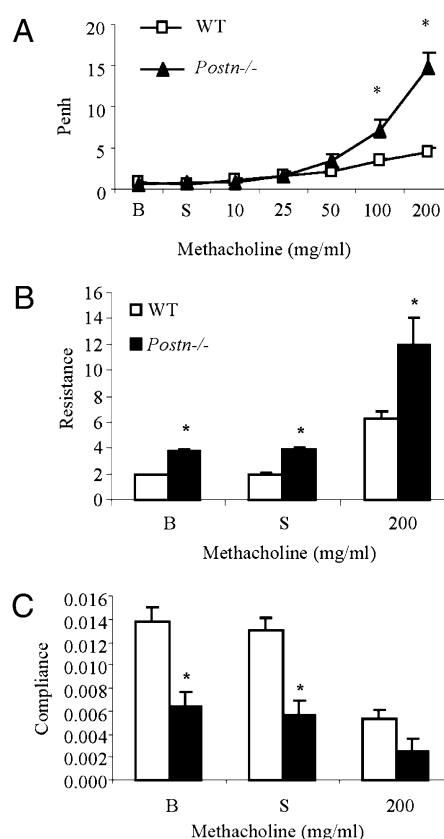


FIGURE 4. Effect of periostin deficiency on AHR in OVA-sensitized and challenged mice. AHR was measured 24 h after the last allergen challenge. A, AHR to aerosolized methacholine was measured in unrestrained, conscious mice by whole body plethysmograph, with mice treated, first with saline, and then with increasing doses, from 10 to 200 mg/ml, of nebulized methacholine (2 min each nebulization). Readings of breathing parameters were taken for 5 min after each nebulization, and Penh was determined by averaging values over the time span. Data represent mean $\pm$ SE from five mice and are representative of three experiments. * p < 0.05, significantly different from WT mice. B and C, Airway resistance and compliance were determined by anesthetizing mice, intubating the trachea, and delivering nebulized saline, followed by doses of methacholine from 5 to 200 mg/ml. Airway resistance and compliance to intratracheal methacholine were determined. Results are representative of two experiments.

3B). Similarly cytokines in the BAL fluid also remained unaltered in mice with a deficiency of periostin (Fig. 3C, *left panel*). However, when BAL cells were stimulated ex-vivo, a significant increase in IL-5 and IL-13 production following anti-CD3 stimulation and IL-5 production following OVA stimulation was observed in cultures from *Postn*^{-/-} mice (Fig. 3C, *middle and right panels*). Moreover, there were increased amounts of Th2 cytokine mRNA isolated from *Postn*^{-/-} lung tissue, compared with littermate controls (Fig. 3D). These results indicate that periostin deficiency does not alter the inflammatory cell populations in the lung but that the cells present in the BAL and lung have modestly increased Th2 cytokine production, compared with littermate controls.

Increased AHR in periostin-deficient mice

To determine the effects of periostin-deficiency on lung function, we assessed lung function using both noninvasive whole-body plethysmography (measured as Penh) and an invasive method of determining airway resistance. In NS and challenged mice, there was no significant difference in Penh or airway resistance between WT and *Postn*^{-/-} mice (data not shown). However, following sensitization and challenge, a significant increase in Penh was observed in *Postn*^{-/-} mice at higher concentrations of metha-

choline (Fig. 4A). Airway resistance using tracheal intubation was increased at baseline, and was also significantly increased at the highest dose of methacholine (Fig. 4B) concomitant with decreased compliance (Fig. 4C). Thus, in sensitized and challenged *Postn*^{-/-} mice, there is increased airway resistance and decreased compliance, compared with control mice, despite a similar recruitment of inflammatory cells to the lungs in mice of both genotypes.

Absence of periostin enhances peripheral immune responses in a model of allergic airway inflammation

To determine whether increased Th2 cytokines in BAL and lungs was a result of increased Th2 responses in the periphery, we examined the cytokine production from cells in the lung draining lymph nodes and spleens. The production of Th2 cytokines IL-4, IL-5, and IL-13 in both splenocyte and mediastinal lymph node cultures was increased following stimulation with anti-CD3 or OVA from cultures of *Postn*^{-/-} cells, compared with WT cells (Fig. 5A, 5B). In contrast, IFN- γ or IL-17 remained unaltered in sensitized and challenged *Postn*^{-/-} (data not shown). Serum OVA-specific IgE levels in *Postn*^{-/-} mice were also significantly increased compared with controls (data not shown). These results suggest that periostin deficiency results in enhanced Th2 responses in vivo.

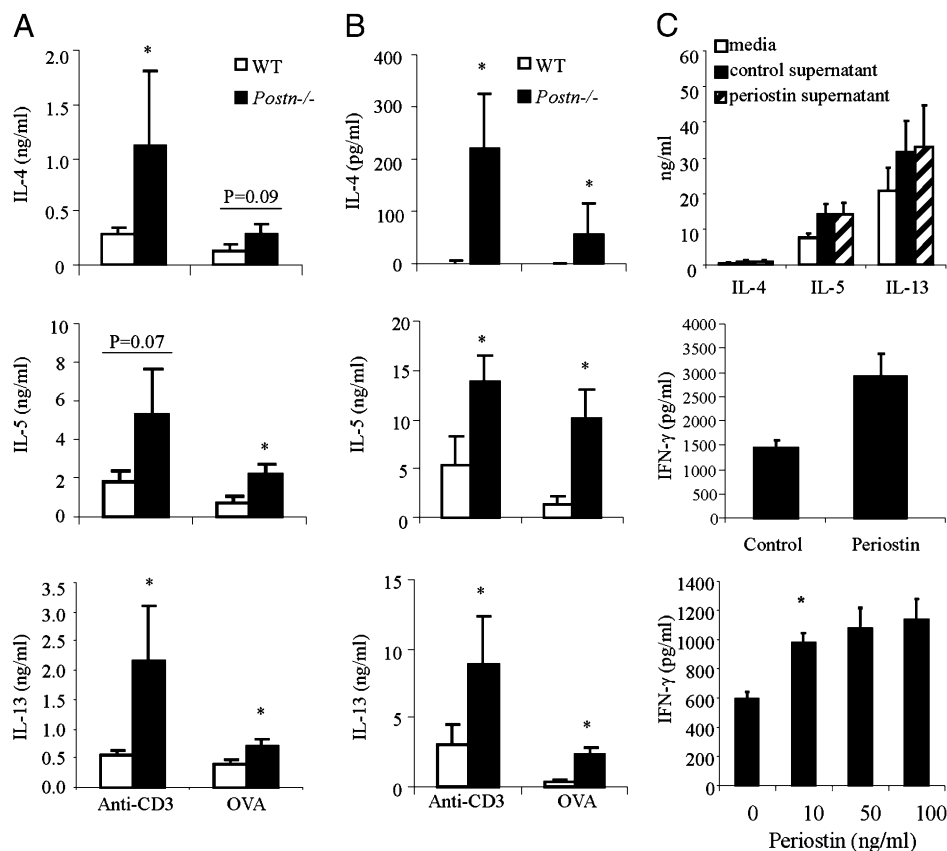


FIGURE 5. Effect of periostin-deficiency on peripheral T cell responses. Splenocytes (A) and mediastinal lymph nodes (B) from OVA sensitized and challenged mice were stimulated with anti-CD3 (4 μ g/ml) or OVA (100 μ g/ml) for 3 d. The levels of IL-4, IL-5, and IL-13 in cell-free supernatants were determined by ELISA. Statistics were performed by a two-tailed *t* test and *p* values for comparisons with WT are indicated. **p* < 0.05, significantly different from WT. C. CD4⁺ T cells were cultured under Th2 (IL-4 + anti-IFN- γ) priming conditions in the presence of periostin or control conditioned media (as described in *Materials and Methods*) for 5 d. Cells were restimulated, and 24-h supernatants were analyzed for cytokine content by ELISA. The levels of IL-4, IL-5, and IL-13 for differentiated cells are shown. Data are expressed as mean $\pm$ SE of three to four WT or *Postn*^{-/-} mice and representative of three experiments (*top panel*). Cultures of CD11c⁺ DC from WT mice were stimulated with LPS and periostin or control media for 72 h and supernatants were tested for the levels of IFN- γ (*middle panel*). CD11c⁺ DC from WT mice were stimulated with LPS and various concentrations of recombinant periostin ranging from 0 to 100 ng/ml and IFN- γ levels measured in 72-h culture supernatants. Data are expressed as the mean $\pm$ SE of three mice (*bottom panel*).

To define a mechanism for how periostin regulated Th2 development we tested the ability of periostin to alter Th2 cell development in vitro. As noted previously, we did not observe any effects of periostin-deficiency on the acquisition of Th phenotypes in vitro (Fig. 1) consistent with a lack of detectable periostin expression in T cells (data not shown). Moreover, addition of periostin-containing media to differentiating T cells did not have an effect on Th2 cytokine production (Fig. 5C, *top panel*). Because periostin did not have any direct effects on T cells, we determined whether periostin could mediate its effects by acting on DC. Addition of periostin-containing media (Fig. 5C, *middle panel*) or recombinant periostin (Fig. 5C, *bottom panel*) to LPS stimulated CD11c⁺ DC increased the levels of IFN- γ . These results suggest that periostin might enhance Th1 responses and that increased Th2 responses develop in the absence of periostin.

GCM in periostin-deficient mice

GCM and mucus overproduction are predominant features of asthma and correlate with AHR resulting in increased severity and mortality of asthmatic patients (28–33). To determine whether mucus production is affected following sensitization and challenge of *Postn*^{-/-} mice, lung tissues were stained with PAS to analyze mucus-producing cells. An increase in mucus production by goblet cells of *Postn*^{-/-} mice was observed (Fig. 6A). Mucus production was quantified by blinded automated measurement of the intensity of PAS staining of goblet cells on three random low power fields per mouse, using a macro developed with the Metamorph software. A significant increase in bronchial PAS staining index was observed in *Postn*^{-/-} mice relative to WT mice (Fig. 6B). Further analysis of mucin gene expression demonstrated significant enhancement of *Muc5ac* and *Gob5* expression in lung tissues of *Postn*^{-/-} mice compared with WT littermates (Fig. 6C). Thus, periostin deficiency markedly augmented mucin gene expression and GCM in the lung.

To directly examine the ability of periostin to inhibit GCM, we tested the effect of periostin on MTEC. Tracheal epithelial cells were isolated and cultured as described in *Materials and Methods*. After 2 d, the confluent cells were stimulated with IL-13 to verify its ability to induce GCM. A 40-fold increase in the expression of *Gob5* at 1 ng/ml IL-13 was observed (Fig. 6D). We next determined the effect of varying doses of periostin (10–100 ng/ml) on IL-13-induced *Gob5* expression in MTEC. A consistent decrease in the expression of IL-13-induced *Gob5* was observed upon treatment with increasing doses of recombinant periostin (Fig. 6D). Thus, periostin may regulate GCM by directly acting on airway epithelial cells.

Discussion

Mucus hypersecretion, a hallmark of asthma is responsible for the increasing morbidity and mortality associated with this disease. However, the underlying mechanisms of mucus production are still poorly understood. Earlier studies have defined a critical role for IL-13 in the induction of GCM. Periostin, an extracellular matrix protein is induced in epithelial cells by inflammatory cytokines including IL-4 and IL-13 (12) and in lung fibroblasts by the anti-inflammatory cytokine TGF- β (34). Moreover, periostin can also colocalize with other extracellular matrix proteins to trigger sub-epithelial fibrosis (12). Whether these effects are important in the development of allergic inflammation was not well characterized. Thus, in the current study, we determined whether periostin promotes inflammation or has a protective homeostatic effect in regulating airway inflammation. Our study demonstrated that periostin-deficiency led to a marked increase in airway resistance and mucus production upon sensitization and challenge with OVA.

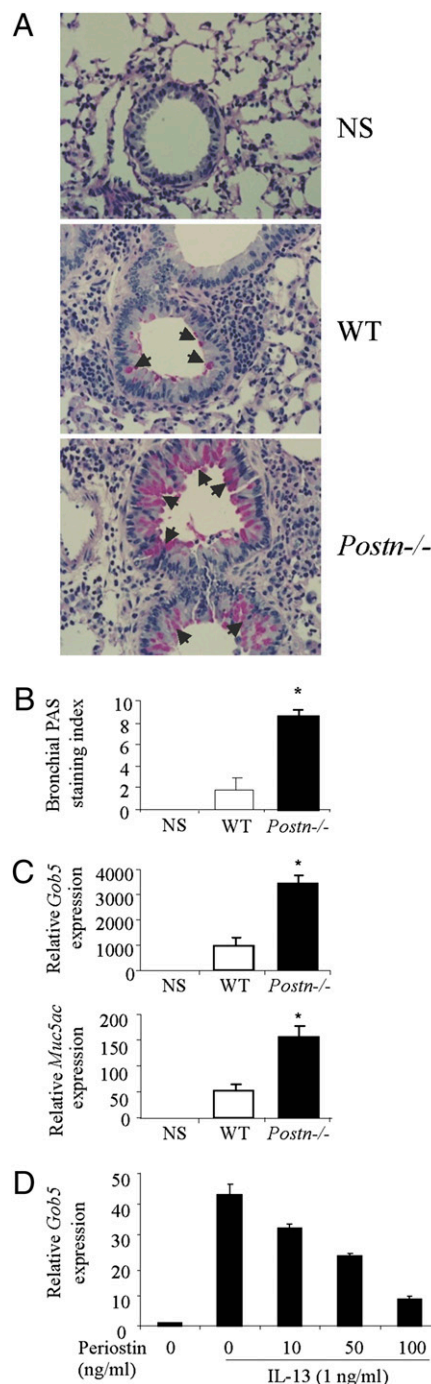


FIGURE 6. Effect of periostin-deficiency on mucus secretion. **A**, Mucus production by goblet cells in airways of NS, OVA-sensitized and challenged WT and *Postn*^{-/-} mice. Lung tissues were fixed and stained with PAS. Representative photomicrographs of $\times 400$ magnification are depicted. Arrows indicate PAS-positive goblet cells. **B**, The extent of mucus production based on PAS staining, measured on coded slides using a macro developed in Metamorph software. Data are expressed as mean $\pm$ SE. * $p < 0.05$, significantly different from WT using a two-tailed Student *t* test. **C**, Mucin gene expression was analyzed from lungs of OVA-sensitized and challenged, WT and *Postn*^{-/-} mice by real-time PCR for *Muc5ac* and *Gob5*. Data are expressed as mean $\pm$ SE, $n = 5$. * $p < 0.05$, significantly different from WT using a two-tailed Student *t* test. **D**, Effect of periostin on IL-13-induced *Gob5* expression. Confluent MTEC were treated with various concentrations of periostin and 1 ng/ml IL-13 for 6 h. Total RNA was extracted and *Gob5* mRNA measured by real-time PCR. Data are representative of three independent experiments.

Despite the development of eosinophilic airway inflammation to a similar extent in *Postn*^{-/-} mice as WT littermates, deficiency of periostin specifically augmented mucus production as evident from PAS-staining index and expression of *Muc5ac* and *Gob5* in allergen-challenged lung tissues.

Recent studies have implicated periostin as a biomarker for IL-13-dependent corticosteroid responsive asthma (35), expressed in airway epithelial cells of asthma patients. Phenotypic heterogeneity of asthmatics could be classified into “Th2-high” and “Th2-low” asthma based on the epithelial expression of the IL-13-inducible genes *POSTN*, *CLCA1*, and *SERPINE2* (36). Patients with “Th2-high asthma” had significantly increased AHR, eosinophilic inflammation, and induction of epithelial mucins *MUC5AC* and *MUC2*. There are similar associations of increased periostin expression in biopsies of patients with EE (20, 37). However, these correlations do not determine the biological function of periostin, which could be acting to promote inflammation, or as part of a negative feedback mechanism to control inflammation. Our studies suggest that allergen sensitization and challenge induced periostin expression (Fig. 2), which functioned to attenuate mucus gene expression and GCM. The mechanism of inhibition of GCM by periostin remains to be elucidated. Earlier studies have suggested that periostin acts as a cell adhesion molecule by binding to integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in cancer cells (38). Thus, it is possible that periostin binds to integrins α_4 and $\beta_{1/2}$, which are involved in asthma (39–48), and may trigger intracellular signaling pathways that repress mucus-inducing transcription factors, including NF- κ B, Sp1, and AP-1 (49). Regardless, our data demonstrate that in the absence of periostin, there was increased differentiation of epithelial cells into mucus-producing goblet cells, suggesting that periostin may contribute to the homeostasis of GCM during allergic inflammation.

How periostin contributes to airway function is still unclear. There were no differences in cellular infiltration, Penh, or airway resistance and compliance, between WT and *Postn*^{-/-} mice in the absence of sensitization and challenge, suggesting that the differences observed in Fig. 4 arise from the allergic inflammation and not from a requirement for periostin in lung development. This is consistent with the observation of low periostin expression in non-challenged lungs (Fig. 2). Analysis of sensitized and challenged mice by whole body plethysmography suggested an increase in AHR. However, use of the more invasive method of testing lung function demonstrated increased airway resistance and decreased compliance in *Postn*^{-/-} mice compared with control mice, even in the absence of methacholine challenge (Fig. 4). Although the airway resistance was significantly increased in *Postn*^{-/-} mice compared with WT mice following methacholine challenge, the fold difference between WT and *Postn*^{-/-} mice at baseline and after methacholine challenge was similar. These results suggest that periostin deficiency is not affecting AHR per se but rather affects baseline lung function following the induction of allergic inflammation. We did not observe obvious changes in epithelial size, smooth-muscle content, or fibrosis between WT and *Postn*^{-/-} lungs, suggesting that the primary effect of periostin deficiency is in increased mucus production that results in decreased airflow through the respiratory tract.

The increased Th2 responses in the periphery were somewhat surprising, considering the lack of a direct effect of periostin on Th cell differentiation. As a possible mechanism for this phenomenon, we demonstrated that periostin augments the production of IFN- γ , a cytokine that both inhibits Th2 and promotes Th1 responses. We propose a model wherein DCs in the lung would be exposed to periostin induced during allergic inflammation that would alter the amounts of cytokines they secrete, when as mature DC, they

migrate to lymph nodes to activate T cells. The ability of periostin to alter Th2 cytokine production might also impact GCM. Thus, the effects of periostin-deficiency on GCM could be through at least two mechanisms; direct effects on airway epithelial cells, and indirect effects of altering APC function and Th2 cytokine production. Although Th2 cytokine production was modestly increased in the lung of *Postn*^{-/-} compared with WT mice, neither inflammation nor *Tslp* expression were appreciably increased (Fig. 3; data not shown). It is possible that the modest increase in Th2 cytokines was not sufficient to alter the amount of inflammation. It is also possible that, as previously reported (20), periostin plays a role in cell recruitment and this was balanced by increased Th2 cytokines. Further studies can distinguish these possibilities.

In a recent study (20) of *Postn*^{-/-} mice, a decrease in eosinophil recruitment to the lungs and increase in blood eosinophilia was observed with no significant differences in mucus production upon intranasal inhalation with allergens from *Aspergillus fumigatus*. There may be several reasons for these differences, including the allergen used to generate airway inflammation. It is also possible that genetic background may contribute to the observed differences; the mice used in our study were on a mixed background, the mice in the previous study, although not stated specifically, were likely C57BL/6 mice (50). Differences in immune pathways activated by these two protocols may result in differential dependence on periostin for regulation of GCM.

In conclusion, our studies have defined a novel previously unrecognized role for periostin in regulation of GCM. Regulation of mucus production is important in minimizing the symptoms and reducing mortality associated with severe asthma attacks (51–53). Our data suggest that although periostin is induced by cytokines associated with allergic inflammation, it functions in the lung to limit mucus production and the resulting effects on airway resistance. This negative feedback mechanism may be an important component of maintaining airway function while the immune system combats pathogens, or in the case of allergens, responds to innocuous proteins. Our results examining the function of periostin in vivo and on primary tracheal epithelial cells suggest that it may be a potential therapeutic in the regulation of mucus production.

Disclosures

The authors have no financial conflicts of interest.

References

- Cohn, L., J. S. Tepper, and K. Bottomly. 1998. IL-4-independent induction of airway hyperresponsiveness by Th2, but not Th1, cells. *J. Immunol.* 161: 3813–3816.
- Vargaftig, B. B., and M. Singer. 2003. Leukotrienes, IL-13, and chemokines cooperate to induce BHR and mucus in allergic mouse lungs. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 284: L260–L269.
- Vignola, A. M., J. Kips, and J. Bousquet. 2000. Tissue remodeling as a feature of persistent asthma. *J. Allergy Clin. Immunol.* 105: 1041–1053.
- Holgate, S. T., M. Peters-Golden, R. A. Panettieri, and W. R. Henderson, Jr. 2003. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J. Allergy Clin. Immunol.* 111: S18–S34; discussion S34–S36.
- Dabbagh, K., K. Takeyama, H. M. Lee, I. F. Ueki, J. A. Lausier, and J. A. Nadel. 1999. IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo. *J. Immunol.* 162: 6233–6237.
- Kuperman, D. A., X. Huang, L. L. Koth, G. H. Chang, G. M. Dolganov, Z. Zhu, J. A. Elias, D. Sheppard, and D. J. Erle. 2002. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat. Med.* 8: 885–889.
- Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C. L. Karp, and D. D. Donaldson. 1998. Interleukin-13: central mediator of allergic asthma. *Science* 282: 2258–2261.
- Louahed, J., M. Toda, J. Jen, Q. Hamid, J. C. Renaud, R. C. Levitt, and N. C. Nicolaides. 2000. Interleukin-9 upregulates mucus expression in the airways. *Am. J. Respir. Cell Mol. Biol.* 22: 649–656.
- Whittaker, L., N. Niu, U. A. Temann, A. Stoddard, R. A. Flavell, A. Ray, R. J. Homer, and L. Cohn. 2002. Interleukin-13 mediates a fundamental pathway

- for airway epithelial mucus induced by CD4 T cells and interleukin-9. *Am. J. Respir. Cell Mol. Biol.* 27: 593–602.
10. Izuhara, K., S. Ohta, H. Shiraishi, S. Suzuki, K. Taniguchi, S. Toda, T. Tanabe, M. Yasuo, K. Kubo, T. Hoshino, and H. Aizawa. 2009. The mechanism of mucus production in bronchial asthma. *Curr. Med. Chem.* 16: 2867–2875.
 11. Yuyama, N., D. E. Davies, M. Akaiwa, K. Matsui, Y. Hamasaki, Y. Suminami, N. L. Yoshida, M. Maeda, A. Pandit, J. L. Lordan, et al. 2002. Analysis of novel disease-related genes in bronchial asthma. *Cytokine* 19: 287–296.
 12. Takayama, G., K. Arima, T. Kanaji, S. Toda, H. Tanaka, S. Shoji, A. N. McKenzie, H. Nagai, T. Hotokebuchi, and K. Izuhara. 2006. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J. Allergy Clin. Immunol.* 118: 98–104.
 13. Takeshita, S., R. Kikuno, K. Tezuka, and E. Amann. 1993. Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. *Biochem. J.* 294: 271–278.
 14. Horiuchi, K., N. Amizuka, S. Takeshita, H. Takamatsu, M. Katsuura, H. Ozawa, Y. Toyama, L. F. Bonewald, and A. Kudo. 1999. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor β . *J. Bone Miner. Res.* 14: 1239–1249.
 15. Zinn, K., L. McAllister, and C. S. Goodman. 1988. Sequence analysis and neuronal expression of fasciclin I in grasshopper and *Drosophila*. *Cell* 53: 577–587.
 16. Terasaka, K., R. Yamaguchi, K. Matsuo, A. Yamazaki, S. Nagai, and T. Yamada. 1989. Complete nucleotide sequence of immunogenic protein MPB70 from *Mycobacterium bovis* BCG. *FEMS Microbiol. Lett.* 49: 273–276.
 17. Skonier, J., M. Neubauer, L. Madisen, K. Bennett, G. D. Plowman, and A. F. Purchio. 1992. cDNA cloning and sequence analysis of β ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor- β . *DNA Cell Biol.* 11: 511–522.
 18. Huber, O., and M. Sumper. 1994. Algal-CAMs: isoforms of a cell adhesion molecule in embryos of the alga *Volvox* with homology to *Drosophila* fasciclin I. *EMBO J.* 13: 4212–4222.
 19. Litvin, J., A. H. Selim, M. O. Montgomery, K. Lehmann, M. C. Rico, H. Devlin, D. P. Bednarik, and F. F. Safadi. 2004. Expression and function of periostin-isoforms in bone. *J. Cell. Biochem.* 92: 1044–1061.
 20. Blanchard, C., M. K. Mingler, M. McBride, P. E. Putnam, M. H. Collins, G. Chang, K. Stringer, J. P. Abonia, J. D. Molkentin, and M. E. Rothenberg. 2008. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. *Mucosal Immunol.* 1: 289–296.
 21. Rios, H., S. V. Koushik, H. Wang, J. Wang, H. M. Zhou, A. Lindsley, R. Rogers, Z. Chen, M. Maeda, A. Kruzynska-Freitag, et al. 2005. periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. *Mol. Cell. Biol.* 25: 11131–11144.
 22. van Rijt, L. S., H. Kuipers, N. Vos, D. Hijdra, H. C. Hoogsteden, and B. N. Lambrecht. 2004. A rapid flow cytometric method for determining the cellular composition of bronchoalveolar lavage fluid cells in mouse models of asthma. *J. Immunol. Methods* 288: 111–121.
 23. Mathur, A. N., H. C. Chang, D. G. Zisoulis, R. Kapur, M. L. Belladonna, G. S. Kansas, and M. H. Kaplan. 2006. T-bet is a critical determinant in the instability of the IL-17-secreting T-helper phenotype. *Blood* 108: 1595–1601.
 24. Giordano, R. J., J. Lahdenranta, L. Zhen, U. Chukwueke, I. Petrache, R. R. Langley, I. J. Fidler, R. Pasqualini, R. M. Tuder, and W. Arap. 2008. Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse. *J. Biol. Chem.* 283: 29447–29460.
 25. Sehra, S., Y. Yao, M. D. Howell, E. T. Nguyen, G. S. Kansas, D. Y. Leung, J. B. Travers, and M. H. Kaplan. 2010. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J. Immunol.* 184: 3186–3190.
 26. Chang, H. C., S. Zhang, V. T. Thieu, R. B. Slee, H. A. Bruns, R. N. Larabee, M. J. Klemsz, and M. H. Kaplan. 2005. PU.1 expression delineates heterogeneity in primary Th2 cells. *Immunity* 22: 693–703.
 27. You, Y., E. J. Richer, T. Huang, and S. L. Brody. 2002. Growth and differentiation of mouse tracheal epithelial cells: selection of a proliferative population. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 283: L1315–L1321.
 28. Aikawa, T., S. Shimura, H. Sasaki, M. Ebina, and T. Takishima. 1992. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* 101: 916–921.
 29. Busse, W. W., R. L. Coffman, E. W. Gelfand, A. B. Kay, and L. J. Rosenwasser. 1995. Mechanisms of persistent airway inflammation in asthma: a role for T cells and T-cell products. *Am. J. Respir. Crit. Care Med.* 152: 388–393.
 30. Carroll, N., S. Carello, C. Cooke, and A. James. 1996. Airway structure and inflammatory cells in fatal attacks of asthma. *Eur. Respir. J.* 9: 709–715.
 31. Fahy, J. V., D. B. Corry, and H. A. Boushey. 2000. Airway inflammation and remodeling in asthma. *Curr. Opin. Pulm. Med.* 6: 15–20.
 32. Laitinen, A., and L. A. Laitinen. 1994. Pathology of asthma. *Allergy Proc.* 15: 323–328.
 33. Shimura, S., Y. Andoh, M. Haraguchi, and K. Shirato. 1996. Continuity of airway goblet cells and intraluminal mucus in the airways of patients with bronchial asthma. *Eur. Respir. J.* 9: 1395–1401.
 34. Oku, E., T. Kanaji, Y. Takata, K. Oshima, R. Seki, S. Morishige, R. Imamura, K. Ohtsubo, M. Hashiguchi, K. Osaki, et al. 2008. Periostin and bone marrow fibrosis. *Int. J. Hematol.* 88: 57–63.
 35. Woodruff, P. G., H. A. Boushey, G. M. Dolganov, C. S. Barker, Y. H. Yang, S. Donnelly, A. Ellwanger, S. S. Sidhu, T. P. Dao-Pick, C. Pantaja, et al. 2007. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc. Natl. Acad. Sci. USA* 104: 15858–15863.
 36. Woodruff, P. G., B. Modrek, D. F. Choy, G. Jia, A. R. Abbas, A. Ellwanger, L. L. Koth, J. R. Arron, and J. V. Fahy. 2009. Th2-driven inflammation defines major subphenotypes of asthma. [Published erratum appears in 2009 *Am. J. Respir. Crit. Care Med.* 180: 796.] *Am. J. Respir. Crit. Care Med.* 180: 388–395.
 37. Blanchard, C., N. Wang, K. F. Stringer, A. Mishra, P. C. Fulkerson, J. P. Abonia, S. C. Jameson, C. Kirby, M. R. Konikoff, M. H. Collins, et al. 2006. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J. Clin. Invest.* 116: 536–547.
 38. Gillan, L., D. Matei, D. A. Fishman, C. S. Gerbin, B. Y. Karlan, and D. L. Koth. 2002. Periostin secreted by epithelial ovarian carcinoma is a ligand for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins and promotes cell motility. *Cancer Res.* 62: 5358–5364.
 39. Borchers, M. T., J. Crosby, S. Farmer, J. Sypek, T. Ansary, N. A. Lee, and J. J. Lee. 2001. Blockade of CD49d inhibits allergic airway pathologies independent of effects on leukocyte recruitment. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 280: L813–L821.
 40. Chin, J. E., C. A. Hatfield, G. E. Winterrowd, J. R. Brashler, S. L. Vonderfecht, S. F. Fidler, R. L. Griffin, K. P. Kolbasa, R. F. Krzesicki, L. M. Sly, et al. 1997. Airway recruitment of leukocytes in mice is dependent on α_4 -integrins and vascular cell adhesion molecule-1. *Am. J. Physiol.* 272: L219–L229.
 41. Henderson, W. R., Jr., E. Y. Chi, R. K. Albert, S. J. Chu, W. J. Lamm, Y. Rochon, M. Jonas, P. E. Christie, and J. M. Harlan. 1997. Blockade of CD49d (α_4 integrin) on intrapulmonary but not circulating leukocytes inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. *J. Clin. Invest.* 100: 3083–3092.
 42. Koo, G. C., K. Shah, G. J. Ding, J. Xiao, R. Wnek, G. Doherty, X. C. Tong, R. B. Pepinsky, K. C. Lin, W. K. Hagmann, et al. 2003. A small molecule very late antigen-4 antagonist can inhibit ovalbumin-induced lung inflammation. *Am. J. Respir. Crit. Care Med.* 167: 1400–1409.
 43. Laberge, S., H. Rabb, T. B. Issekutz, and J. G. Martin. 1995. Role of VLA-4 and LFA-1 in allergen-induced airway hyperresponsiveness and lung inflammation in the rat. *Am. J. Respir. Crit. Care Med.* 151: 822–829.
 44. Nakajima, H., H. Sano, T. Nishimura, S. Yoshida, and I. Iwamoto. 1994. Role of vascular cell adhesion molecule 1/very late activation antigen 4 and intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 interactions in antigen-induced eosinophil and T cell recruitment into the tissue. *J. Exp. Med.* 179: 1145–1154.
 45. Lee, S. H., J. E. Prince, M. Rais, F. Kheradmand, F. Shardonofsky, H. Lu, A. L. Beaudet, C. W. Smith, L. Soong, and D. B. Corry. 2003. Differential requirement for CD18 in T-helper effector homing. *Nat. Med.* 9: 1281–1286.
 46. Kanwar, S., C. W. Smith, F. R. Shardonofsky, and A. R. Burns. 2001. The role of Mac-1 (CD11b/CD18) in antigen-induced airway eosinophilia in mice. *Am. J. Respir. Cell Mol. Biol.* 25: 170–177.
 47. Schneider, T., T. B. Issekutz, and A. C. Issekutz. 1999. The role of α_4 (CD49d) and β_2 (CD18) integrins in eosinophil and neutrophil migration to allergic lung inflammation in the Brown Norway rat. *Am. J. Respir. Cell Mol. Biol.* 20: 448–457.
 48. Banerjee, E. R., Y. Jiang, W. R. Henderson, Jr., Y. Latchman, and T. Papayanopoulou. 2009. Absence of α_4 but not β_2 integrins restrains development of chronic allergic asthma using mouse genetic models. *Exp. Hematol.* 37: 715–727.e3.
 49. Thai, P., A. Loukoianov, S. Wachi, and R. Wu. 2008. Regulation of airway mucin gene expression. *Annu. Rev. Physiol.* 70: 405–429.
 50. Oka, T., J. Xu, R. A. Kaiser, J. Melendez, M. Hambleton, M. A. Sargent, A. Lorts, E. W. Brunskill, G. W. Dorn, II, S. J. Conway, et al. 2007. Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. *Circ. Res.* 101: 313–321.
 51. Rogers, D. F. 2004. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr. Opin. Pharmacol.* 4: 241–250.
 52. Rose, M. C., and J. A. Voynow. 2006. Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol. Rev.* 86: 245–278.
 53. Fahy, J. V. 2002. Goblet cell and mucin gene abnormalities in asthma. *Chest* 122 (Suppl. 6):320S–326S.