

Periostin is required for maximal airways inflammation and hyperresponsiveness in mice

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Background: Periostin, a secreted extracellular matrix protein, has been localized to deposits of subepithelial fibrosis in asthmatic patients, and periostin levels have been linked to increases in IL-13.

Objective: We hypothesized that periostin is required for airway inflammatory responses to a physiologic aeroallergen, house dust mite (HDM).

Methods: We studied F4-F6 B6;129-*Postn*^{tm1Jmol}/J wild-type (*Postn*^{+/+}) and null (*Postn*^{-/-}) mice, as well as C57BL/6 mice treated with either IgM or OC-20 periostin neutralizing antibody. Mice were exposed to 5 doses of HDM intranasally over a 16-day period.

Results: HDM increased airways responsiveness in *Postn*^{+/+} but not *Postn*^{-/-} mice. In addition, HDM-treated C57BL/6 mice injected with OC-20 had lower airways responsiveness than HDM-treated mice injected with IgM. Compared with *Postn*^{+/+} mice, *Postn*^{-/-} mice showed decreases in HDM-induced inflammation and mucous metaplasia, as well as reduced IL-4, IL-25, CD68, Gob5, and periostin mRNA expression. OC-20 antibody produced similar results. HDM exposure increased periostin expression in the airway epithelium, subepithelium, smooth muscle and inflammatory cells. OC-20 blocked the HDM-induced IgE response, and T cells incubated with dendritic cells (DCs) from *Postn*^{-/-} mice or treated with OC-20 showed deficient DNA synthesis and IL-13 responses compared with T cells incubated with wild-type DCs. Finally, adoptive transfer of bone marrow-derived DCs from *Postn*^{+/+} mice was sufficient to promote allergic responses in F6 *Postn*^{-/-} littermates.

Conclusions: In mice, periostin is required for maximal airways hyperresponsiveness and inflammation after HDM sensitization and challenge. Periostin is required for maximal HDM-induced T-cell responses. (J Allergy Clin Immunol 2014;134:1433-42.)

Key words: Periostin, asthma, mouse models, inflammation, matricellular protein, house dust mite, integrin, CD11b

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Abbreviations used

BAL: Bronchoalveolar lavage
BrdU: Bromodeoxyuridine
DC: Dendritic cell
HDM: House dust mite
OVA: Ovalbumin
PAS: Periodic acid-Schiff
Postn: Periostin
qPCR: Quantitative PCR
TCR: T-cell receptor

Matricellular proteins are nonstructural proteins that are secreted and sequestered in the extracellular matrix, where they interact with integrins, growth factors, proteases, cytokines, and other extracellular matrix proteins. One matricellular protein, periostin, is deposited in the airway subepithelium of asthmatic patients.¹ Periostin mRNA expression is also increased in the airway epithelium, and expression levels correlate with disease severity.² Treatment with the anti-IL-13 antibody lebrikizumab improves lung function in asthmatic patients, and patients with high pretreatment levels of serum periostin had greater improvement in lung function than patients with low periostin levels.³ In patients with persistent symptoms despite inhaled corticosteroids, the serum periostin level is the single best predictor of airway eosinophilia.⁴ Periostin is a marker of omalizumab responsiveness in patients with uncontrolled severe persistent allergic asthma.⁵ Recently, it was shown in asthmatic patients that high serum periostin levels (≥ 95 ng/mL) were associated with a greater decrease in FEV₁ per year.⁶ These data are consistent with the notion that periostin plays a deleterious role in asthma pathogenesis.

The periostin gene encodes a 93-kDa glycoprotein comprised of an N-terminal secretory signal sequence and 4 fasciclin domains. Periostin also undergoes alternative splicing in its C-terminal region,⁷ leading to smaller isoforms. Periostin interacts with other extracellular matrix proteins and is a ligand for $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_4\beta_6$, and $\alpha_M\beta_2$ (CD11b) integrins. For example, eosinophils adhere to periostin through CD11b,⁸ and periostin increases eosinophil adhesion to fibronectin.⁹ Periostin also regulates TGF- β signaling¹⁰ and promotes myofibroblast differentiation of mesenchymal cells.¹¹⁻¹³ Because periostin has the potential to regulate both adhesion of lung inflammatory cells and mesenchymal cell differentiation, we hypothesized that periostin is required for maximal allergic airways disease in mice.

METHODS

Mice

C57BL/6 and F2 B6;129-*Postn*^{tm1Jmol}/J mice¹⁴ were purchased from the Jackson Laboratory (Bar Harbor, Me). Homozygous *Postn*^{-/-} mice

were backcrossed into the C57BL/6 strain for 2 to 4 additional generations (F4-F6). Most experiments compared F4 or F6 homozygous *Postn*^{-/-} mice with their homozygous *Postn*^{+/+} littermates. The remainder of the experiments, examining the effects of an anti-periostin neutralizing antibody (see below), were conducted in C57BL/6 mice. Genotyping was performed by Transnetx (Cordova, Tenn) and verified by using specific primers and quantitative PCR (qPCR) assays.

Models of allergic airways disease

We exposed 8- to 12-week-old C57BL/6 and F4-F6 B6;129 *Postn*^{+/+} and *Postn*^{-/-} mice to 100 μ g of *Dermatophagoides pteronyssinus* house dust mite (HDM) extract in 50 μ L of PBS (Greer Laboratories, Lenoir, NC) by means of intranasal installation on days 0, 7, 14, 15, and 16. Mice were anesthetized with isoflurane for each treatment. Animals were studied on day 17. Alternatively, mice were exposed to LPS-free ovalbumin (OVA; Pierce, Rockford, Ill), as previously described.¹⁵ Briefly, mice received intraperitoneal injections of 20 μ g of OVA in 2 mg of alum on days 0 and 7 and 100 μ g of intranasal OVA on days 14 through 19. Mice were killed on day 21. Changes in airways resistance to nebulized methacholine were assessed in anesthetized tracheotomized mice by using a Buxco FinePointe plethysmograph (Wilmington, NC).¹⁶

Periostin neutralization

Mice were injected intraperitoneally with 200 μ g of OC-20 mouse anti-periostin mAb (Sirius-Biotech, Genoa, Italy) on days 7 and 14 of HDM exposure. OC-20 blocks periostin's interaction with the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins.^{13,17}

Analysis of airway inflammation

Lungs sections were stained with hematoxylin and eosin or periodic acid-Schiff (PAS) reagent to detect mucins. Bronchoalveolar lavage (BAL) leukocyte differential counts were performed, as previously described.¹⁸

Harvesting of lung tissue for flow cytometry, qPCR, and immunostaining

For flow cytometry, cell pellets were resuspended in serum containing medium with BSA, anti-mouse CD16/32 (BioLegend, San Diego, Calif) and fluorescent antibody or matched isotype control.^{19,20} Cells were analyzed on a FACSCanto 2 (BD Biosciences, San Jose, Calif) with FACSDiva (BD Biosciences) and FlowJo (Tree Star, Ashland, Ore) software. Up to 10⁵ cells were analyzed per sample. CD45, CD11b, CD11c, F4/80 (BioLegend), Siglec-F (eBioscience, San Diego, Calif), and Gr1 (R&D Systems, Minneapolis, Minn) levels were monitored.

Aliquots were also taken for RNA extraction with Trizol (Invitrogen, Grand Island, NY). Poly-A RNA was purified (RNeasy Plus Mini kit; Qiagen, Valencia, Calif), and first-strand cDNA was produced for quantitative 2-step real-time PCR (Realplex2; Eppendorf, Westbury, NY). Primer sequences used are shown in Table 1. Results were normalized against glyceraldehyde-3-phosphate dehydrogenase.

For fluorescence microscopy, sections were probed with fluorescence-labeled mouse anti- α -smooth muscle actin (clone 1A4; Sigma-Aldrich, St Louis, Mo), polyclonal rabbit anti-periostin (Abcam, Cambridge, Mass), anti-I-A/I-E (mouse MHC class II, BioLegend), or specific IgG or IgM isotype controls. For immunohistochemistry, sections were probed with rabbit anti-periostin and stained with a biotinylated anti-rabbit IgG-avidin horseradish peroxidase and diaminobenzidine detection system (Vector Laboratories, Burlingame, Calif).

Measurement of serum IgE

IgE was assayed by means of ELISA (BioLegend).

Requirement of periostin for dendritic cell activation

To determine whether periostin is required for dendritic cell (DC) activation, we used an *in vitro* assay examining the response of bone

marrow-derived DCs to HDM *in vitro*, with T-cell IL-13 expression and bromodeoxyuridine (BrdU) incorporation as outputs.²¹ Bone marrow was harvested from 2 F4 or F6 *Postn*^{-/-} mice and 2 *Postn*^{+/+} littermates. For differentiation, DCs were cultured for 7 days with 10% FBS, 40 ng/mL GM-CSF, and 15 ng/mL IL-4. On day 7, cells were pulsed with PBS or 100 μ g/mL HDM in PBS with either 100 μ g/mL mouse IgM or OC-20 anti-periostin antibody. For selected experiments, 1×10^5 allogeneic T cells, purified from BALB/cJ mouse spleens by using the MACS T Cell Isolation Kit (Miltenyi Biotec, Auburn, Calif), were added on day 8 of the protocol. On day 10, cells were harvested; stained for dead cells with a Pacific orange viability dye; incubated with Pacific blue-conjugated anti-CD83, fluorescein isothiocyanate-conjugated anti-CD80, Alexa Fluor 555-conjugated anti-IA-IE (a mouse MHC2 protein), phycoerythrin-Cy5-conjugated anti-CD45, allophycocyanin-conjugated anti-CD11c, and allophycocyanin-Cy7-conjugated anti-CD86; and subjected to flow cytometry. Cells were stimulated with brefeldin A, ionomycin, and phorbol 12-myristate 13-acetate for 4 hours to assess IL-13 production. Cells were incubated with fluorescent anti-T-cell receptor (TCR) β and anti-CD45 (BioLegend), fixed, permeabilized, incubated with labeled anti-IL-13 (eBioscience), and harvested for flow cytometry. IL-13 in cell supernatants was assessed by means of ELISA (eBioscience). To measure T-cell DNA synthesis, 1 μ mol/L BrdU (Sigma-Aldrich) was added 4 hours before harvest on day 10, and cells were labeled with labeled anti-BrdU (BioLegend).

Adoptive transfer

F6 *Postn*^{+/+} and *Postn*^{-/-} littermate bone marrow cells were differentiated and matured with HDM, as above. Next, 1×10^6 labeled cells in 50 μ L of PBS were administered intratracheally to anesthetized *Postn*^{-/-} mice. On day 11, sensitization and challenge with HDM or PBS was begun. On day 28, lungs were harvested and processed for PAS staining.

RESULTS

Periostin is required for allergen-induced airways inflammation and mucous metaplasia

F4 B6;129 *Postn*^{+/+} and *Postn*^{-/-} mice¹⁴ were exposed to 5 intranasal exposures of HDM over a 17-day period. Immunohistochemical staining showed that HDM exposure increased periostin expression in the airway epithelium, subepithelium, and inflammatory cell infiltrates (Fig 1, A-D). Periostin expression was absent in *Postn*^{-/-} mice. Fluorescence microscopy showed colocalization of periostin and α -smooth muscle actin in the airway smooth muscle layer (Fig 1, E-H). Finally, immunofluorescence showed reduced periostin expression in OC-20-treated mice (Fig 1, I-K). Sections were stained with hematoxylin and eosin or PAS reagent to detect mucus. HDM-exposed *Postn*^{+/+} mice showed substantial peribronchial inflammation and mucous metaplasia (Fig 2, A and B). Compared with *Postn*^{+/+} mice, *Postn*^{-/-} mice showed decreased inflammation and mucous metaplasia (Fig 2, C and D).

We also exposed F6 B6;129 *Postn*^{+/+} and *Postn*^{-/-} littermates to OVA sensitization and challenge. OVA-exposed *Postn*^{+/+} mice showed peribronchial inflammation and mucous metaplasia, although less than seen in HDM-treated mice. *Postn*^{-/-} littermates showed decreased inflammation and mucous metaplasia (Fig 2, E and F).

We also examined the requirement for periostin in HDM-exposed C57BL/6 mice using a mouse neutralizing mAb (OC-20).^{13,17} OC-20 blocked HDM-induced inflammation and mucous metaplasia (Fig 3).

We examined the effects of periostin deficiency on inflammatory cells in BAL fluid and lung tissue. HDM exposure increased neutrophil, macrophage/monocyte, lymphocyte, and eosinophil

TABLE I. Primer sequences used for qPCR

Gene	Forward primer	Reverse primer
<i>Il4</i>	5'-TGC ACC ATG AAT GAG TCC AA-3'	5'-GGT GGC TCA GTA CTA CGA GTA ATC-3'
<i>Il13</i>	5'-GGT CTT GTG TGA TGT TGC TCA-3'	5'-CCT GGC TCT TGC TTG CCT T-3'
<i>Il10</i>	5'-GCT CTT ACT GAC TGG CAT GAG-3'	5'-CGC AGC TCT AGG AGC ATG TG-3'
<i>Il17a</i>	5'-GCC TGA GAG CTG CCC CTT CAC-3'	5'-GGC TGC CTG GCG GAC AAT CG-3'
<i>Il25</i>	5'-ACA GGG ACT TGA ATC GGG TC-3'	5'-TGG TAA AGT GGG ACG GAG TTG-3'
<i>Cd68</i>	5'-TCC CAA GAG CCC CCT TGG ACC-3'	5'-TCC ACT GTT GGC CCT CAC CCT-3'
<i>Gob5</i>	5'-CTG TCT TCC TCT TGA TCC TCC A-3'	5'-CGT GGT CTA TGG CGA TGA CG-3'
<i>Muc5b</i>	5'-GAG CAG TGG CTA TGT GAA AAT CAG-3'	5'-CAG GGC GCT GTC TTC TTC AT-3'
<i>Postn</i>	5'-GCC TTA GCG ACC TCT ACA AT-3'	5'-TAG CCG TCC GAT ACA CAA-3'
<i>Cyr61</i>	5'-CCA TGG CCA GAA ATG CAT CG-3'	5'-AAC TCG TGT GGA GAT GCC AG-3'
<i>Gapdh</i>	5'-GTC GGT GTG AAC GGA TTT G-3'	5'-GTC GTT GAT GGC AAC AAT CTC-3'

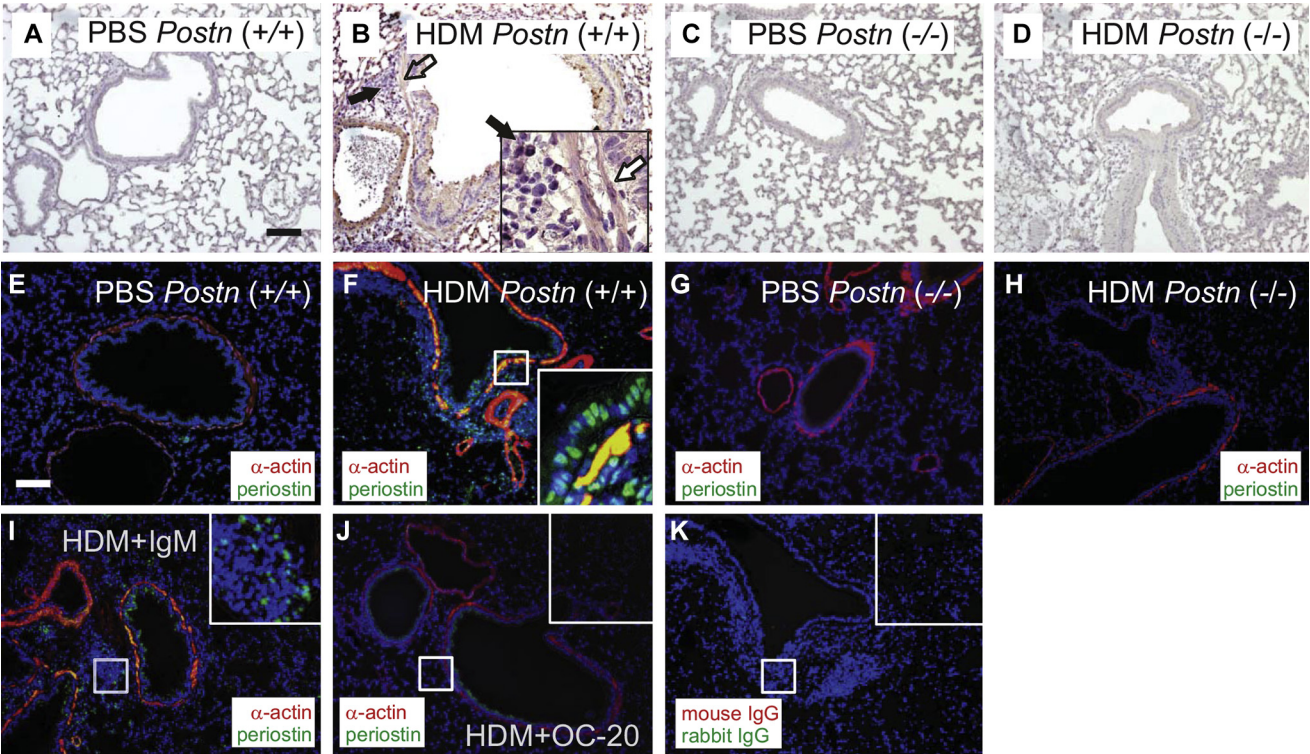


FIG 1. Periostin localization in airways of HDM-treated mice. **A–D**, Periostin staining in the lungs of PBS-treated (Fig 1, A and C) and HDM-treated (Fig 1, B and D) mice. Lungs from F4 *Postn*^{+/+} (Fig 1, A and B) and *Postn*^{-/-} mice (Fig 1, C and D) are shown. Fig 1, B, Periostin-positive airway smooth muscle (white arrow) and inflammatory cells (black arrow). Images are shown at ×100 magnification, except for the ×400 magnification inset. Bars = 100 μm. **E–K**, Immunofluorescence for α-actin (red) and periostin (green), with colocalization in yellow and nuclei in blue. Fig 1, E, PBS-treated *Postn*^{+/+} mouse. Fig 1, F, HDM-treated *Postn*^{+/+} mouse. Fig 1, G, PBS-treated *Postn*^{-/-} mouse. Fig 1, H, HDM-treated *Postn*^{-/-} mouse. Fig 1, I, C57BL/6 mouse treated with HDM and IgM. Fig 1, J, C57BL/6 mouse treated with HDM and OC-20. Fig 1, K, Control slide stained with mouse IgG and rabbit IgG. These figures are representative of 3 separate experiments.

numbers in BAL fluid. Compared with *Postn*^{+/+} mice, *Postn*^{-/-} mice showed reductions in each cell type (Fig 4, A). IgM-treated HDM-exposed C57BL/6 mice also showed increases in BAL inflammatory cell counts, although the changes were less marked (Fig 4, B). OC-20-treated mice showed significant reductions in BAL fluid eosinophil counts. Next, we examined lung inflammatory cells using flow cytometry. Compared with HDM-exposed *Postn*^{+/+} mice, *Postn*^{-/-} mice had fewer lung Gr1⁺ neutrophils, TCRβ⁺ T cells, and Gr1⁺ and Siglec-F⁺ eosinophils (Fig 4, C–F).

Compared with HDM-exposed IgM-treated C57BL/6 mice, OC-20-treated mice had fewer lung T cells, neutrophils, and eosinophils.

Periostin is required for HDM-induced airways hyperresponsiveness

Changes in airways cholinergic responsiveness were assessed, as described above. HDM increased responsiveness in F4 B6;129 *Postn*^{+/+} mice (Fig 4, G). In contrast, the airways responsiveness of

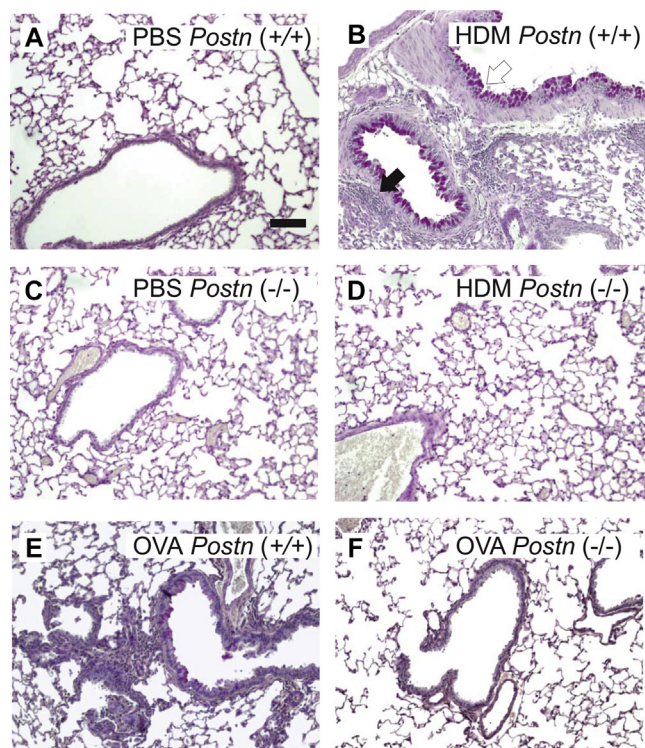


FIG 2. HDM treatment increases mucus production and airway inflammation in *Postn*^{+/+} mice but not *Postn*^{-/-} mice. **A-D**, Lung sections from F4 B6;129 *Postn*^{+/+} (Fig 2, A and B) or *Postn*^{-/-} mice (Fig 2, C and D) treated with PBS (Fig 2, A and C) or HDM (Fig 2, B and D) were stained with PAS and hematoxylin. Note the magenta PAS staining (open arrow) and hematoxylin-stained inflammatory cells (black arrow). **E and F**, Lung sections from F6 B6;129 *Postn*^{+/+} (Fig 2, E) or *Postn*^{-/-} (Fig 2, F) mice treated with OVA. Original magnification is $\times 100$ X. Bar = 100 μ m. These figures are representative of 3 separate experiments.

HDM-exposed littermate *Postn*^{-/-} mice was not different from that seen in PBS control animals. We also examined the requirement for periostin in HDM-exposed IgM- and OC-20-treated C57BL/6 mice. HDM-treated C57BL/6 mice injected intraperitoneally with OC-20 had a significantly lower methacholine response than HDM-treated control animals injected with IgM (Fig 4, H).

Periostin-deficient mice show lower HDM-induced lung cytokine mRNA expression

Lung cytokine mRNA was monitored by using qPCR. Compared with HDM-treated F4 B6;129 *Postn*^{+/+} mice, *Postn*^{-/-} mice showed significant reductions in mRNAs encoding IL-4, IL-10, IL-13, IL-17a, IL-25, CD68 (a macrophage marker), Gob5 (a chloride channel which regulates mucus production), Cyr61 (another matricellular protein), and, as expected, periostin (Fig 5, A). Compared with C57BL/6 mice treated with HDM and IgM, mice treated with HDM and the neutralizing anti-periostin OC-20 mAb showed reductions in IL-13, IL-25, CD68, Gob5, and Muc5B, and periostin levels (Fig 5, B). Changes in thymic stromal lymphopoietin levels were not statistically significant.

A neutralizing antibody to periostin decreases serum IgE response to HDM sensitization

We sensitized C57BL/6 mice to HDM in the presence of OC-20 or an IgM control. HDM exposure increased serum IgE levels,

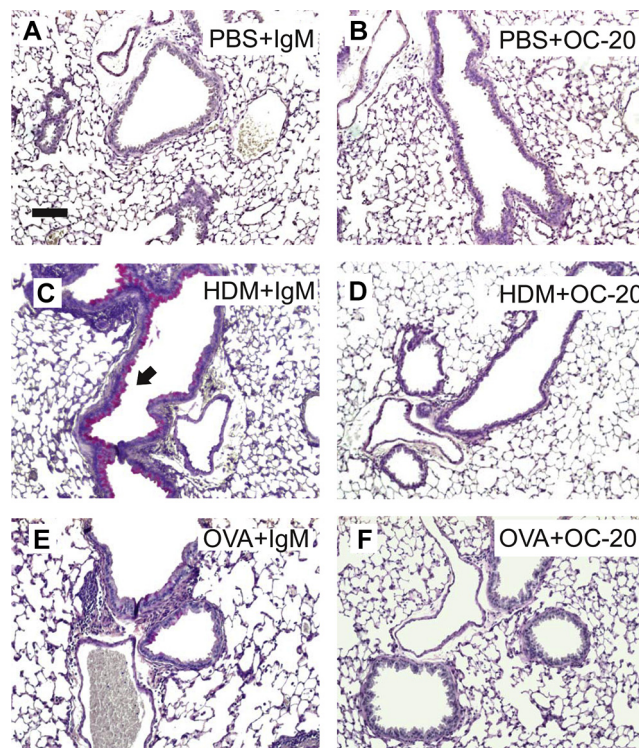


FIG 3. A periostin-neutralizing mAb, OC-20, blocks allergen-induced mucus production and airway inflammation in C57BL/6 mice. **A-D**, PAS-stained sections from the lungs of PBS-exposed (Fig 3, A and B) or HDM-exposed (Fig 3, C and D) C57BL/6 mice treated on days 7 and 14 with 200 μ g IgM (Fig 3, A and C) or 200 μ g of OC-20 (Fig 3, B and D). **E and F**, Mouse lung sections from OVA-exposed C57BL/6 mice treated with IgM (Fig 3, E) or OC-20 (Fig 3, F). These figures are representative of 3 separate experiments.

and OC-20 blocked the IgE response (Fig 6, A). These data suggest that periostin is required for maximal HDM-induced type 2 immune responses.

Periostin-deficient mice show incomplete DC maturation and deficient T-cell activation

We hypothesized that CD11b⁺ DCs require interactions with periostin to facilitate T-cell activation and T_H2 differentiation. To determine whether periostin is required for maximal DC function, we used an *in vitro* assay examining the response of bone marrow-derived DCs to HDM *in vitro* using T-cell IL-13 expression as an output.²¹

First, we sorted live CD45⁺CD83⁺CD11c⁺IA-IE⁺ cells for CD80 and CD86 (Fig 6, B and C). Only *Postn*^{+/+} DCs incubated without OC-20 expressed both CD80 and CD86. Next, we examined the number of IL-13-expressing T cells. When bone marrow-derived DCs from *Postn*^{+/+} mice were used, the HDM pulse increased the number of IL-13⁺TCR β ⁺ cells (Fig 6, D and F). There was no increase when DCs from periostin knockout mice were used. Similarly, incubation with OC-20, but not IgM, blocked DC activation of T-cell IL-13 production. When we measured IL-13 levels using ELISA, we only detected IL-13 production by wild-type allogeneic T cells incubated with HDM-pulsed DCs (45.1 ± 7.6 pg/mL, $n = 3$). On the other hand, knockout and OC-20-treated DCs did not activate T-cell IL-13 production. Finally, HDM-pulsed DCs from

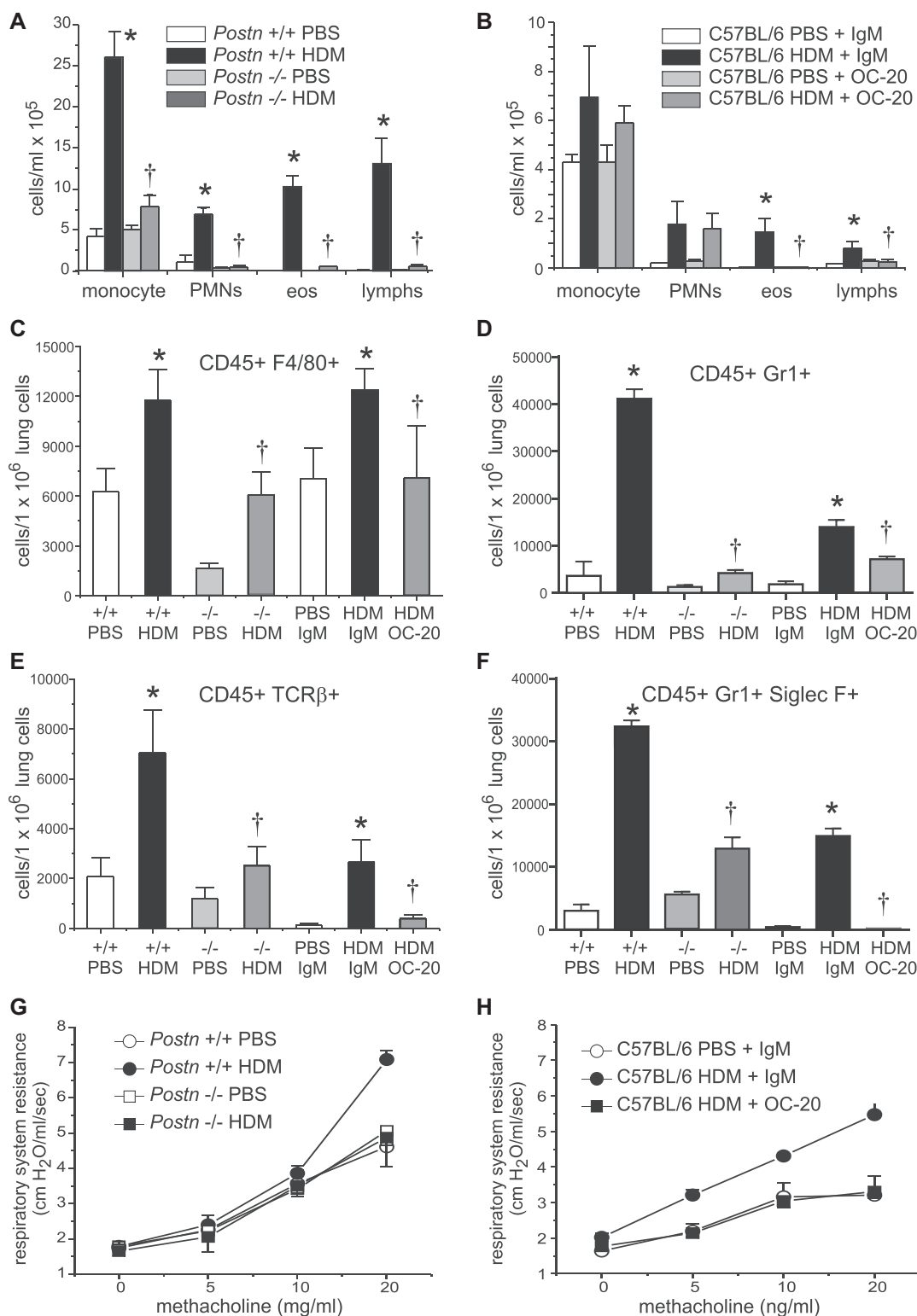


FIG 4. Periostin is required for HDM-induced airways inflammation and hyperresponsiveness. **A**, BAL cells in HDM-treated F4 B6;129 *Postn*^{+/+} mice (open bars) and *Postn*^{-/-} littermates (black bars; n = 4). *Different from *Postn*^{+/+} PBS mice and †different from *Postn*^{+/+} HDM mice (*P* < .05, 1-way ANOVA). **B**, HDM-exposed C57BL/6 mice were injected with IgM or OC-20 (n = 4). *Different from PBS plus IgM mice and †different from HDM plus IgM mice (*P* < .05, 1-way ANOVA). **C-F**, Lung cells interacted with antibodies against CD45, F4/80 (Fig 4, C), TCRβ (Fig 4, D), Gr1 (Fig 4, E), or both Gr1 and Siglec-F (Fig 4, F; n = 6-12). *Different from *Postn*^{+/+} PBS mice or C57BL/6 PBS plus IgM mice, as appropriate (*P* < .05). †Different from *Postn*^{+/+} HDM mice or C57BL/6 HDM plus IgM mice (*P* < .05, 1-way ANOVA). **G**, Airways responsiveness was higher for HDM-exposed F4 B6;129 *Postn*^{+/+} mice than *Postn*^{-/-} littermates (n = 6). **P* < .05, 2-way ANOVA. **H**, Airways responsiveness in HDM-exposed C57BL/6 mice injected with IgM or OC-20 (n = 4). **P* < .05, 2-way ANOVA.

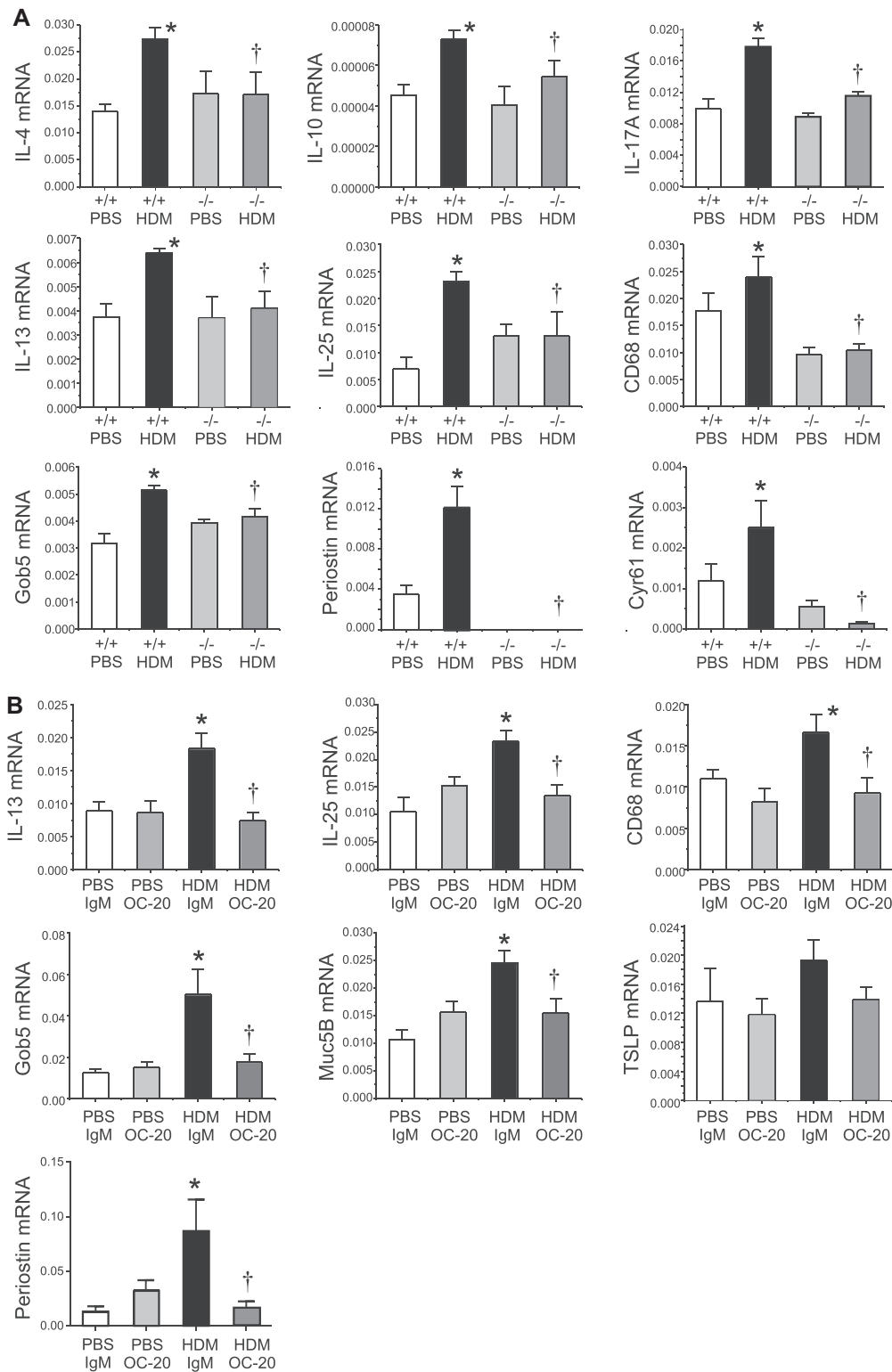


FIG 5. *Postn*^{-/-} and OC-20-treated mice show decreased HDM-induced mRNA expression. **A**, mRNA expression was measured by using qPCR (n = 4). *Different from *Postn*^{+/+} PBS mice ($P < .05$) and †different from *Postn*^{+/+} HDM mice ($P < .05$, 1-way ANOVA). **B**, C57BL/6 mice were treated with HDM or PBS without or with isotypic IgM or OC-20 (n = 4). *Different from PBS plus IgM mice ($P < .05$) and †different from HDM plus IgM mice ($P < .05$, 1-way ANOVA).

Postn^{+/+} mice stimulated BrdU incorporation into allogeneic T cells, whereas HDM-pulsed DCs from *Postn*^{-/-} mice did not (Fig 6, E and G). OC-20 blocked this effect.

To further examine the role of DC periostin expression, we examined the lungs of HDM-exposed *Postn*^{+/+} and *Postn*^{-/-} mice for I-A/I-E (mouse MHC class II) and periostin (Fig 7, A).

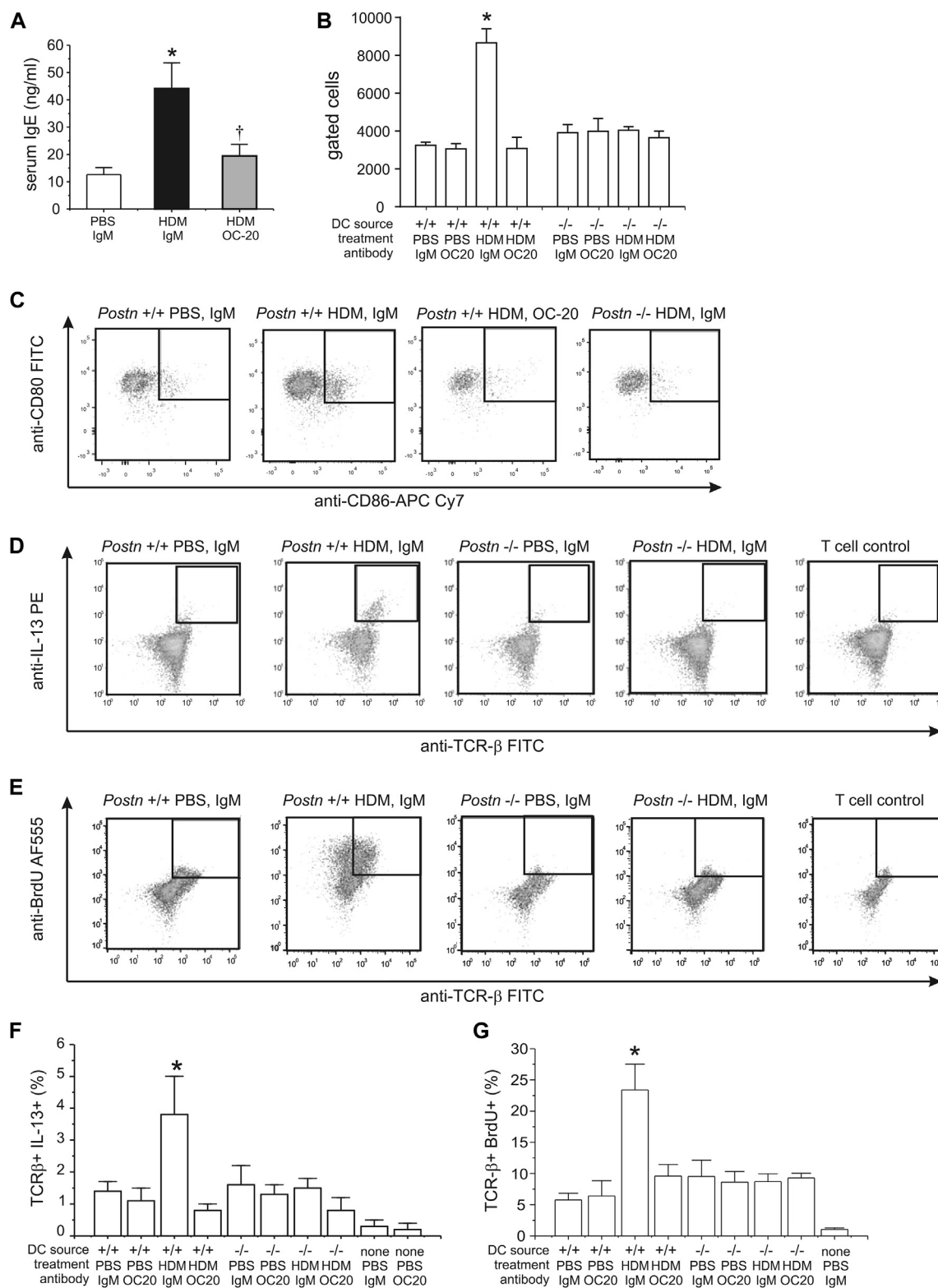


FIG 6. Periostin is required for IgE production, DC CD86 expression, and allogeneic T-cell IL-13 production and DNA synthesis. **A**, Serum IgE levels measured by means of ELISA ($n = 11$). *Different from PBS plus IgM ($P < .05$) and †different from HDM plus IgM ($P < .05$, 1-way ANOVA). **B–G**, Mice were sensitized to HDM, and bone marrow cells from F4 or F6 *Postn* ^{+/+} and *Postn* ^{-/-} littermates were differentiated to DCs, pulsed with PBS or HDM, and incubated with IgM or OC-20. For selected experiments, allogeneic T cells were added. Fig 6, **B**, Group mean data are shown for CD80⁺ CD86⁺ expression by DCs. Fig 6, **C**, CD80 and CD86 expression by live CD45⁺ CD83⁺ IA-IE⁺ CD11c⁺ DCs. Fig 6, **D–H**, DC activation of allogeneic T-cell IL-13 production and BrdU incorporation. Fig 6, **D**, TCRβ and IL-13 levels were assessed by using flow cytometry. Fig 6, **E**, Group mean data for live CD45⁺ TCRβ⁺ IL-13⁺ cells. Fig 6, **F**, DCs and allogeneic T cells were prepared as above, and BrdU was added before harvest on day 10. Fig 6, **G**, Group mean data for the TCRβ⁺ BrdU⁺ cells. Group mean data for Fig 6, **B**, **E**, and **G**, are means $\pm$ SEMs ($n = 3$). *Different from all other groups ($P < .05$, 1-way ANOVA). AF555, Alexa Fluor 555; APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

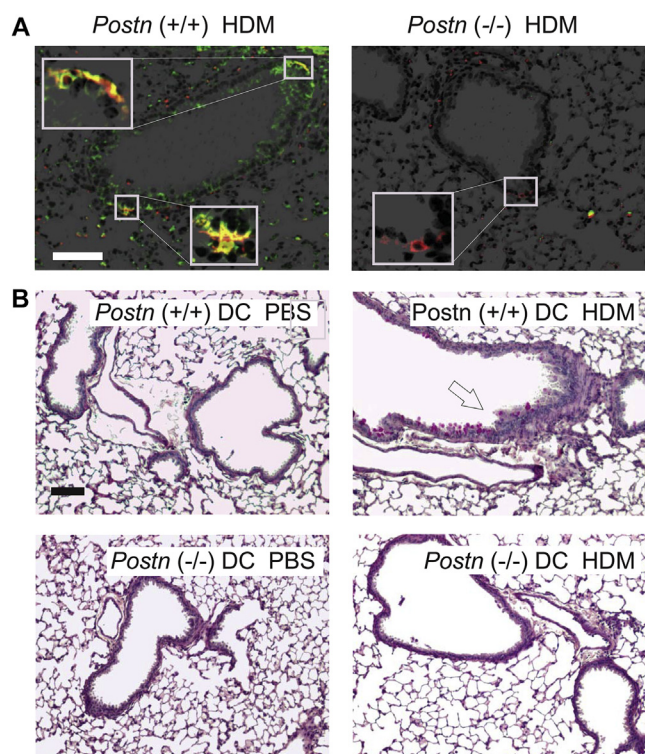


FIG 7. DCs from *Postn*^{+/+} mice are sufficient for allergic airway inflammation. **A**, Lungs from HDM-exposed F6 B6;129 *Postn*^{+/+} mice and *Postn*^{-/-} littermates were immunostained for periostin (green) and I-A/I-E (red), a mouse DC marker (original magnification $\times 100$; inset $\times 800$). **B**, HDM-matured, differentiated, bone marrow-derived DCs from *Postn*^{+/+} or *Postn*^{-/-} mice were transferred to *Postn*^{-/-} mice intratracheally challenged with PBS or HDM. PAS-stained lung sections from PBS-treated and HDM-treated mice treated with *Postn*^{+/+} DCs or *Postn*^{-/-} DCs ($\times 100$ magnification). Bar = 100 μ m. These figures are representative of 3 separate experiments.

I-A/I-E⁺ cells from *Postn*^{+/+} mice expressed periostin. Next, we tested the sufficiency of *Postn*^{+/+} DCs for HDM-induced inflammation by transferring HDM-matured, differentiated, bone marrow-derived DCs from *Postn*^{+/+} to *Postn*^{-/-} mice and then challenging with HDM *in vivo* (Fig 7, B). Mice treated with HDM-matured *Postn*^{+/+} but not *Postn*^{-/-} DCs showed a greater sensitivity to HDM challenge, as assessed based on PAS and hematoxylin staining. These data suggest that periostin-expressing DCs are sufficient for HDM-induced allergic inflammation.

DISCUSSION

We examined the requirement of periostin for HDM-induced airways disease. We found deposition of periostin in airway epithelial, subepithelial, smooth muscle, and inflammatory cells after HDM exposure. HDM sensitization and challenge increased airways cholinergic responsiveness in *Postn*^{+/+} but not *Postn*^{-/-} mice. Similarly, HDM-treated C57BL/6 mice injected with OC-20 had a lower methacholine response than HDM-treated control animals injected with IgM. Compared with *Postn*^{+/+} mice, *Postn*^{-/-} mice showed decreases in HDM-induced inflammation and mucous metaplasia. Exposure to OVA instead of HDM and periostin blockade with a periostin neutralizing antibody each produced comparable results. OC-20 blocked the

HDM-induced IgE response, and DCs isolated from *Postn*^{-/-} mice or treated with OC-20 showed an absence of CD86 expression after HDM exposure. In addition, T cells incubated with DCs isolated from *Postn*^{-/-} mice or treated with OC-20 showed deficient DNA synthesis and IL-13 responses compared with those of T cells incubated with wild-type IgM-treated DCs. Finally, transfer of periostin-expressing DCs to *Postn*^{-/-} mice restored HDM responsiveness *in vivo*. Together, these results indicate that periostin has a proinflammatory effect in the context of allergic airways disease.

As noted above, periostin has been shown to be deposited in the airway tissues of patients with asthma, and expression levels correlate with disease severity.^{1,2} Patients with high pretreatment levels of serum periostin show greater improvement in lung function with lebrikizumab³ or omalizumab.⁵ In patients with uncontrolled asthma, the serum periostin level is the best predictor of airway eosinophilia.⁴ Finally, it was recently shown that polymorphisms of the periostin gene are related to higher serum periostin levels and a more rapid decrease in FEV₁.⁶ These new data are consistent with the notion that periostin is not simply a marker of asthma severity but instead plays a contributing role in asthma pathogenesis. Our data, which show that inhibition of periostin expression/function attenuates allergen-induced airways disease, agree with this concept. Our data are also consistent with previous work using the *Postn*^{-/-} mouse, showing that F2 B6;129 *Postn* knockout mice show reduced eosinophil recruitment to the lungs after *Aspergillus fumigatus* sensitization and challenge.⁹ Periostin has also been shown to promote inflammation in other models of allergic disease, including HDM-induced atopic dermatitis,²² OVA-induced allergic rhinitis,²³ and *A. fumigatus*-induced eosinophilic esophagitis.⁹

Our data starkly contrast with 2 studies in B6;129 periostin-deficient (*peri*^{lacZ}) mice²⁴ showing a protective effect of periostin on allergen-induced airways hyperresponsiveness.^{15,25} Although it is conceivable that extracellular matrix deposition prevents smooth muscle shortening in asthmatic patients, these discrepant results might relate to differences in backcrossing or the mode of allergen administration. Another possibility is that *peri*^{lacZ} knockout mice experience a subtle change in airway wall development, which increases airway collapsibility: although *Postn*^{-/-} homozygotes are slightly smaller than *Postn*^{+/+} and heterozygous animals (not shown), *peri*^{lacZ} mice exhibit dwarfism, enamel defects, and early-onset periodontal disease.²⁴

To address potential issues of knockout mouse strain and backcrossing on the results, we used a periostin neutralizing antibody as a second method of periostin inhibition. This antibody inhibits ovarian cancer growth²⁶ and bleomycin-induced inflammation and fibrosis in mice.¹³ Our studies indicate not only that OC-20 decreases HDM-induced periostin accumulation but also that OC-20 attenuates HDM-induced airways responsiveness and inflammation. These data corroborate our findings in F4 *Postn*^{+/+} and *Postn*^{-/-} mice. Interestingly, OC-20 appeared to decrease periostin mRNA and protein expression. It is possible that neutralization of periostin blocks expression by interrupting an autocrine periostin loop, as has been shown in pancreatic stellate cells and aortic abdominal aneurysm tissue.^{27,28}

We have not completed backcrossing of the *Postn* mice. F6 B6;129-*Postn* knockout mice should contain more than 98% of C57BL/6 DNA, with less than 2% 129S genetic background of varying composition. However, it is possible that adjacent genes

could be responsible for the effects we observed, even with heterozygote mating. Finally, the response of F6 B6;129-*Postn* mice to antigen challenge is unlikely to be due to 129S genetic elements because these mice are capable of having lung allergic inflammatory responses.²⁹

Despite human data suggesting that periostin might play a role in asthma pathogenesis, the mechanisms by which periostin promotes asthmatic airways inflammation and hyperresponsiveness are almost completely unknown. Possibilities include the following: (1) periostin, a ligand for $\alpha_M\beta_2$ (CD11b), augments CD11b⁺ DC activation and T_H2 cell differentiation; (2) periostin serves as a cell-binding matrix, thereby increasing infiltration of inflammatory cells in the airways; and (3) periostin promotes airway smooth muscle hypertrophy and contractile protein expression, increasing contractility and airways responsiveness. On the basis of our results showing reduced IgE levels in periostin-depleted OC-20-treated mice, we hypothesized that CD11b⁺ DCs require interactions with periostin to facilitate T-cell activation and T_H2 differentiation.

CD11b^{high} DCs are responsible for CD4⁺ T_H2 responses to OVA³⁰ and HDM.³¹ We found that DCs from *Postn*^{-/-} mice did not express CD86 after HDM exposure, which, on engagement with CD28, provides CD4⁺ T cells with the costimulatory signal that decreases activation threshold and allows activation.³² In addition, splenic T cells incubated with DCs isolated from *Postn*^{-/-} mice or treated with OC-20 each showed deficient HDM-induced IL-13 responses compared with those seen in T cells incubated with wild-type or IgM-treated DCs. Furthermore, transfer of bone marrow-derived DCs from *Postn*^{+/+} mice were sufficient to promote allergic responses in HDM-exposed *Postn*^{-/-} mice. Together, these results suggest that periostin is required for maximal DC activation. Periostin has been shown to activate nuclear factor κ B in keratinocytes,^{22,33} and it is possible that nuclear factor κ B is involved in DC activation.

In conclusion, we have shown that in mice periostin is required for maximal allergen-induced airway responses. Results were similar in *Postn*^{-/-} mice and mice treated with a neutralizing antibody. Thus periostin might represent a promising therapeutic target in asthmatic patients.

Key messages

- Periostin has been shown to be a marker for severe allergic asthma, but its role in asthma pathogenesis is not clear.
- Using knockout mice and a neutralizing antibody, we show that periostin is required for maximal allergic airways responses in mice and that periostin-positive DCs are sufficient to promote allergic airways disease in *Postn*^{-/-} mice.
- These data indicate that periostin might represent a promising therapeutic target in asthmatic patients.

REFERENCES

1. Takayama G, Arima K, Kanaji T, Toda S, Tanaka H, Shoji S, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 2006;118:98-104.
2. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A* 2007;104:15858-63.
3. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab Treatment in Adults with Asthma. *N Engl J Med* 2011;365:1088-98.
4. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012;130:647-54.e10.
5. Hanania NA, Wenzel S, Rosén K, Hsieh H-J, Mosesova S, Choy DF, et al. Exploring the Effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013;187:804-11.
6. Kanemitsu Y, Matsumoto H, Izuhara K, Tohma 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.e3.
7. Hoersch S, Andrade-Navarro M. Periostin shows increased evolutionary plasticity in its alternatively spliced region. *BMC Evol Biol* 2010;10:30.
8. Johansson MW, Annis DS, Mosher DF. $\alpha_M\beta_2$ integrin-mediated adhesion and motility of interleukin-5-stimulated eosinophils on periostin. *Am J Respir Cell Mol Biol* 2013;48:503-10.
9. Blanchard C, Mingler MK, McBride M, Putnam PE, Collins MH, Chang G, et al. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. *Mucosal Immunol* 2008;1:289-96.
10. Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, et al. Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. *Circ Res* 2008;102:752-60.
11. Vi L, Feng L, Zhu RD, Wu Y, Satish L, Gan BS, et al. Periostin differentially induces proliferation, contraction and apoptosis of primary Dupuytren's disease and adjacent palmar fascia cells. *Exp Cell Res* 2009;315:3574-86.
12. Bozyk PD, Bentley JK, Popova AP, Anyanwu AC, Linn MD, Goldsmith AM, et al. Neonatal periostin knockout mice are protected from hyperoxia-induced alveolar simplification. *PLoS One* 2012;7:e31336.
13. Naik PK, Bozyk PD, Bentley JK, Popova AP, Birch CM, Wilke CA, et al. Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L1046-56.
14. Oka T, Xu J, Kaiser RA, Melendez J, Hambleton M, Sargent MA, et al. Genetic Manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. *Circ Res* 2007;101:313-21.
15. Sehra S, Yao W, Nguyen ET, Ahly A-NN, Barbé Tuana FM, Ahlfeld SK, et al. Periostin regulates goblet cell metaplasia in a model of allergic airway inflammation. *J Immunol* 2011;186:4959-66.
16. Newcomb DC, Sajjan US, Nagarkar DR, Wang Q, Nanua S, Zhou Y, et al. Human rhinovirus 1B exposure induces phosphatidylinositol 3-kinase-dependent airway inflammation in mice. *Am J Respir Crit Care Med* 2008;177:1111-21.
17. Orecchia P, Conte R, Balza E, Castellani P, Borsi L, Zardi L, et al. Identification of a novel cell binding site of periostin involved in tumour growth. *Eur J Cancer* 2011;47:2221-9.
18. Schneider D, Hong JY, Bowman ER, Chung Y, Nagarkar DR, McHenry CL, et al. Macrophage/epithelial cell CCL2 contributes to rhinovirus-induced hyperresponsiveness and inflammation in a mouse model of allergic airways disease. *Am J Physiol Lung Cell Mol Physiol* 2013;304:L162-9.
19. Osterholzer JJ, Chen G-H, Olszewski MA, Curtis JL, Huffnagle GB, Toews GB. Accumulation of CD11b⁺ lung dendritic cells in response to fungal infection results from the CCR2-mediated recruitment and differentiation of Ly-6C^{high} monocytes. *J Immunol* 2009;183:8044-53.
20. Schneider D, Hong JY, Popova AP, Bowman ER, Linn MJ, McLean AM, et al. Neonatal rhinovirus infection induces persistent mucous metaplasia and airways hyperresponsiveness. *J Immunol* 2012;188:2894-904.
21. Lewkowich IP, Lajoie S, Clark JR, Herman NS, Sproles AA, Wills-Karp M. Allergen uptake, activation, and IL-23 production by pulmonary myeloid DCs drives airway hyperresponsiveness in asthma-susceptible mice. *PLoS One* 2008;3:e3879.
22. 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.
23. Hur DG, Khalmuratova R, Ahn S-K, Ha Y-S, Min Y-G. Roles of periostin in symptom manifestation and airway remodeling in a murine model of allergic rhinitis. *Allergy Asthma Immunol Res* 2012;4:222-30.
24. Rios H, Koushik SV, Wang H, Wang J, Zhou H-M, Lindsley A, et al. Periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. *Mol Cell Biol* 2005;25:11131-44.
25. Gordon ED, Sidhu SS, Wang ZE, Woodruff PG, Yuan S, Solon MC, et al. A protective role for periostin and TGF- β in IgE-mediated allergy and airway hyperresponsiveness. *Clin Exp Allergy* 2012;42:144-55.
26. Hart CE, Forstrom JW, Kelly JD, Seifert RA, Smith RA, Ross R, et al. Two classes of PDGF receptor recognize different isoforms of PDGF. *Science* 1988;240:1529-31.

27. Erkan M, Kleeff J, Gorbachevski A, Reiser C, Mitkus T, Esposito I, et al. Periostin creates a tumor-supportive microenvironment in the pancreas by sustaining fibrogenic stellate cell activity. *Gastroenterology* 2007;132:1447-64.
28. Yamashita O, Yoshimura K, Nagasawa A, Ueda K, Morikage N, Ikeda Y, et al. Periostin links mechanical strain to inflammation in abdominal aortic aneurysm. *PLoS One* 2013;8:e79753.
29. Oh S-Y, Zheng T, Bailey ML, Barber DL, Schroeder JT, Kim Y-K, et al. Src homology 2 domain-containing inositol 5-phosphatase 1 deficiency leads to a spontaneous allergic inflammation in the murine lung. *J Allergy Clinical Immunol* 2007;119:123-31.
30. Kinjo Y, Wu D, Kim G, Xing G-W, Poles MA, Ho DD, et al. Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 2005;434:520-5.
31. Mesnil C, Sabatel CM, Marichal T, Toussaint M, Cataldo D, Drion P-V, et al. Resident CD11b⁺Ly6C[−] lung dendritic cells are responsible for allergic airway sensitization to house dust mite in mice. *PLoS One* 2012;7:e53242.
32. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 1996;14:233-58.
33. Nishiyama T, Kii I, Kashima TG, Kikuchi Y, Ohazama A, Shimazaki M, et al. Delayed re-epithelialization in periostin-deficient mice during cutaneous wound healing. *PLoS One* 2011;6:e18410.

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