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BRIEF COMMUNICATION

Cell type-dependent effects of corticosteroid on periostin production by primary human tissue cells

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Abstract

Overproduction of periostin, an IL-13-inducible matricellular protein, despite corticosteroid treatment is thought to be involved in the chronicity of allergic inflammation seen in corticosteroid-refractory tissue fibrosis. Therefore, we hypothesized that some tissue cells must produce periostin in a corticosteroid-insensitive manner. Here, we show that IL-4 and IL-13 each induced comparable levels of periostin production by primary normal human fibroblasts and microvascular endothelial cells derived from lung and skin. Dexamethasone, a corticosteroid, completely inhibited IL-4/13-induced, but did not affect TGF-β-induced, periostin production by fibroblasts. In contrast, dexamethasone synergistically enhanced IL-4/13-induced periostin production by microvascular endothelial cells. TGF-β did not induce periostin production by microvascular endothelial cells. Our novel findings suggest that IL-4/13-induced microvascular endothelium-derived and/or TGF-β-induced fibroblast-derived periostin might play a pivotal role in corticosteroid-refractory tissue fibrosis, leading to chronic allergic inflammation in the lung and/or skin.

Periostin, a matricellular protein originally isolated from an osteoblast cell line (1), is reported to be induced by each of IL-4, IL-13 and TGF-β in airway epithelial cells (2), and lung and skin fibroblasts (3, 4). Increasing evidence indicates that epithelial cell- and/or fibroblast-derived periostin is involved in the pathogenesis of allergic diseases, including asthma and atopic dermatitis, as a component in subepithelial fibrosis (2–4). Furthermore, lebrikizumab, a humanized anti-IL-13 mAb, significantly improved lung function in patients with refractory asthma, especially in serum periostin-high patients (5). Serum periostin levels have also been shown to be a systemic biomarker for development of airflow limitation (6) and airway eosinophilic inflammation (7) in asthmatic patients receiving inhaled corticosteroid (ICS). This clinical evidence strongly suggests that overproduction of periostin despite adequate ICS treatment plays a key role in the chronicity of allergic inflammation seen in corticosteroid-refractory lung fibrosis in asthma. However, an earlier study demonstrated that IL-13-induced periostin expression by airway epithelial cells was completely inhibited by dexamethasone treatment (2). Therefore, we hypothesized that some other tissue cells must produce periostin in a corticosteroid-insensitive manner.

In this study, we investigated the effects of dexamethasone (SIGMA, St. Louis, MO, USA) on IL-4-, IL-13- and TGF-β1- (R&D Systems, Minneapolis, MN, USA) induced production of periostin by primary human tissue cells, including normal human lung fibroblasts (NHLF), human microvascular endothelial cells from lung blood vessels (HMVEC-LBl), normal human dermal fibroblasts (NHDF), and human microvascular endothelial cells from dermal blood vessels (HMVEC-dBl) (Lonza, Walkersville, MD, USA). Cells were treated with 10 ng/ml IL-4, IL-13 or TGF-β in the presence and absence of 100 nM dexamethasone for 48 h. Total RNA extraction, cDNA synthesis, and qPCR were performed as previously described (8). Primer sets for periostin (sense, 5′-CACACCCGTGAGGAAGTTGC-3′; antisense, 5′- TTTCA CTGAGAACGACCTTCCC-3′) and β-actin (sense, 5′-CCC AGCCATGTACGTTGCTAT-3′; antisense, 5′-TCACCGGA

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GTCCATCACGAT-3′) were synthesized at Fasmac (Kanagawa, Japan). Concentrations of periostin in the cell-free supernatants were measured with a specific ELISA kit (R&D Systems) in accordance with the manufacturer's instructions.

As a result, IL-4 and IL-13 each significantly induced comparable levels of periostin production by NHLF, HMVEC-LBl, NHDF and HMVEC-dBl (Fig. 1; upper graphs). Although fibroblasts have been believed to be the primary cellular sources of periostin induced by Th2 cytokines, this is the first study to show that microvascular endothelial cells derived from lung and skin (HMVEC-LBl and HMVEC-dBl) are comparable to fibroblasts (NHLF and NHDF) in their ability to produce periostin protein *in vitro*. Dexamethasone completely inhibited both IL-4- and IL-13-induced periostin production by NHLF and NHDF. Conversely, dexamethasone synergistically enhanced both IL-4- and IL-13-induced periostin production by HMVEC-LBl and HMVEC-dBl. TGF-β also induced periostin production by NHLF and NHDF, whereas dexamethasone did not inhibit their TGF-β-induced periostin production. TGF-β did not induce periostin production by HMVEC-LBl or HMVEC-dBl. All the qPCR data (Fig. 1; lower graphs) fully agreed with the ELISA data (Fig. 1; upper graphs), suggesting that cell type-dependent effects of corticosteroid on periostin production by tissue cells are transcriptionally regulated.

Asthma is now considered a heterogeneous disease, presenting with several distinct phenotypes (9). Woodruff et al. (10) reported that asthma of mild-to-moderate severity can be divided into at least two distinct molecular phenotypes

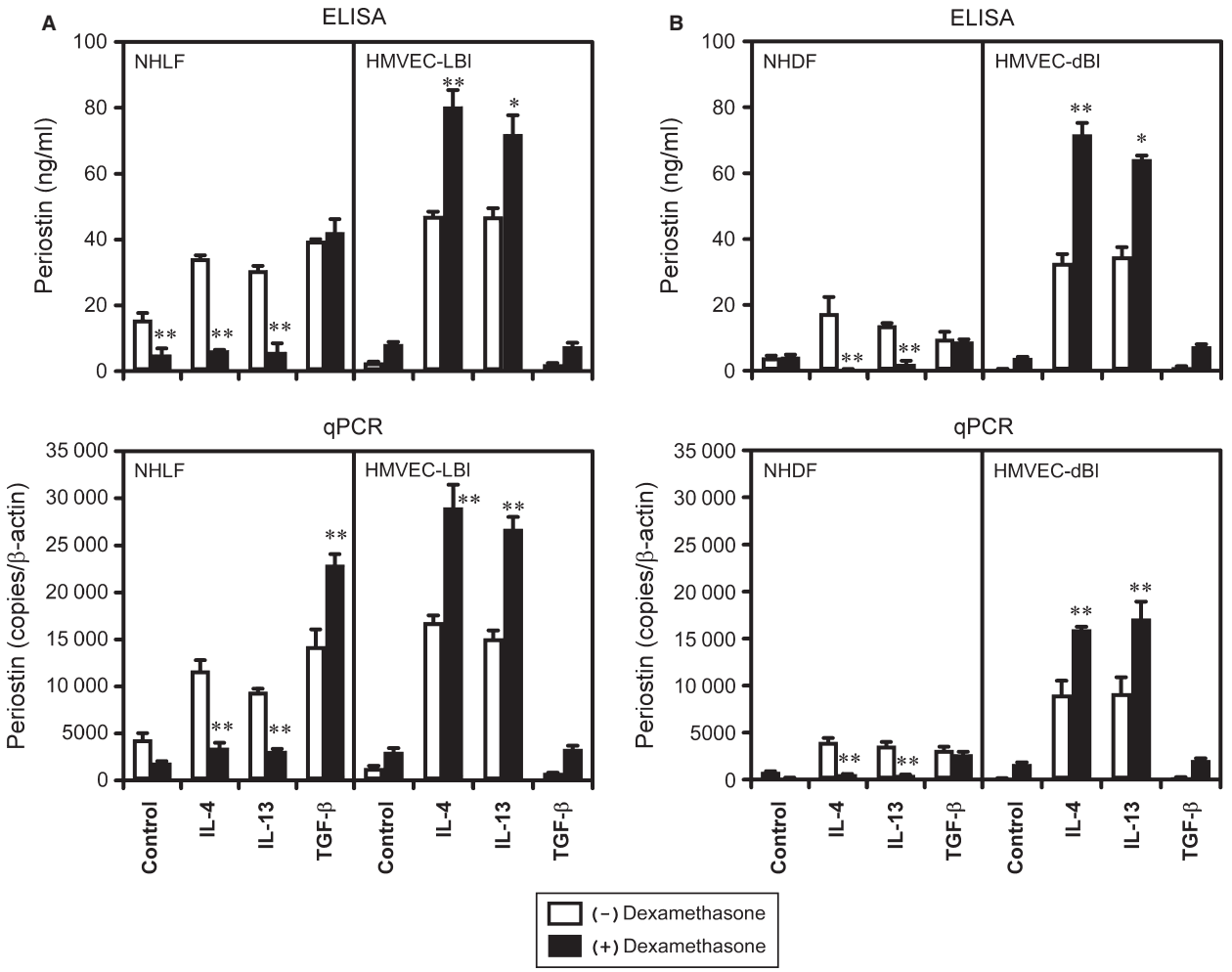


Figure 1 Effects of dexamethasone on IL-4-, IL-13- and TGF-β-induced production of periostin by primary human tissue cells. (A) Lung- and (B) skin-derived human tissue cells were treated with 10 ng/ml IL-4, IL-13 or TGF-β in the presence (closed bars) and absence (open bars) of 100 nM dexamethasone for 48 h. Periostin concentrations in the cell-free culture supernatants (upper graphs) and periostin transcripts (lower graphs) were measured. All data are presented as the mean ± SD of triplicate samples. We performed the same experiments with each type of cells originating from at least two different donors and obtained reproducible results (data not shown). Differences between groups were analyzed using ANOVA with Bonferroni's *post hoc* test. $*P < 0.05$ and $**P < 0.01$ compared with without dexamethasone.

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based on the degree of Th2 inflammation, which is characterized by high levels of IL-13 and periostin mRNA expression in bronchial biopsies. The periostin-high subgroup in mild-to-moderate asthma showed improved lung function following ICS treatment (10). That was probably because ICS suppressed not only IL-13-producing cells (11), but also IL-13-induced periostin production by airway epithelial cells (2) and fibroblasts (Figs 1 and 2A).

Of note, TGF-β-induced periostin production by fibroblasts was not inhibited by dexamethasone treatment (Fig. 1). TGF-β is thought to play a pivotal role in airway remodeling in asthmatic patients, and airway TGF-β1 expression was increased in children with severe atopic asthma despite high-dose ICS treatment (12). Although that study did not evaluate airway or serum periostin levels, TGF-β-induced periostin may be involved in the refractory fibrosis seen in this population. TGF-β did not induce periostin production by microvascular endothelial cells. However, IL-4/13-induced periostin production by those cells was further enhanced by dexamethasone treatment (Fig. 1).

Our data showed good agreement between lung- and skin-derived tissue cells, suggesting that periostin-regulated inflammatory responses would be similar in both lung and skin. Indeed, Masuoka et al. (4) recently demonstrated that periostin plays a key role in the amplification and chronicity of allergic skin inflammation.

Our present findings may help explain how overproduction of periostin is promoted in subepithelial tissue and suggest that IL-4/13-induced microvascular endothelium-derived and/or TGF-β-induced fibroblast-derived periostin might play a pivotal role in corticosteroid-refractory tissue fibrosis, leading to chronic allergic inflammation in the lung and/or skin. Based on our results, we postulate the roles of corticosteroid in the production of periostin in subepithelial tissues (Fig. 2).

RU-486, a corticosteroid receptor antagonist, completely abrogated both the enhancing and inhibitory effects of dexamethasone on periostin production (data not shown), suggesting that dexamethasone acts through corticosteroid receptors to alter periostin expression in tissue cells. Clarifying the precise molecular mechanisms underlying the cell

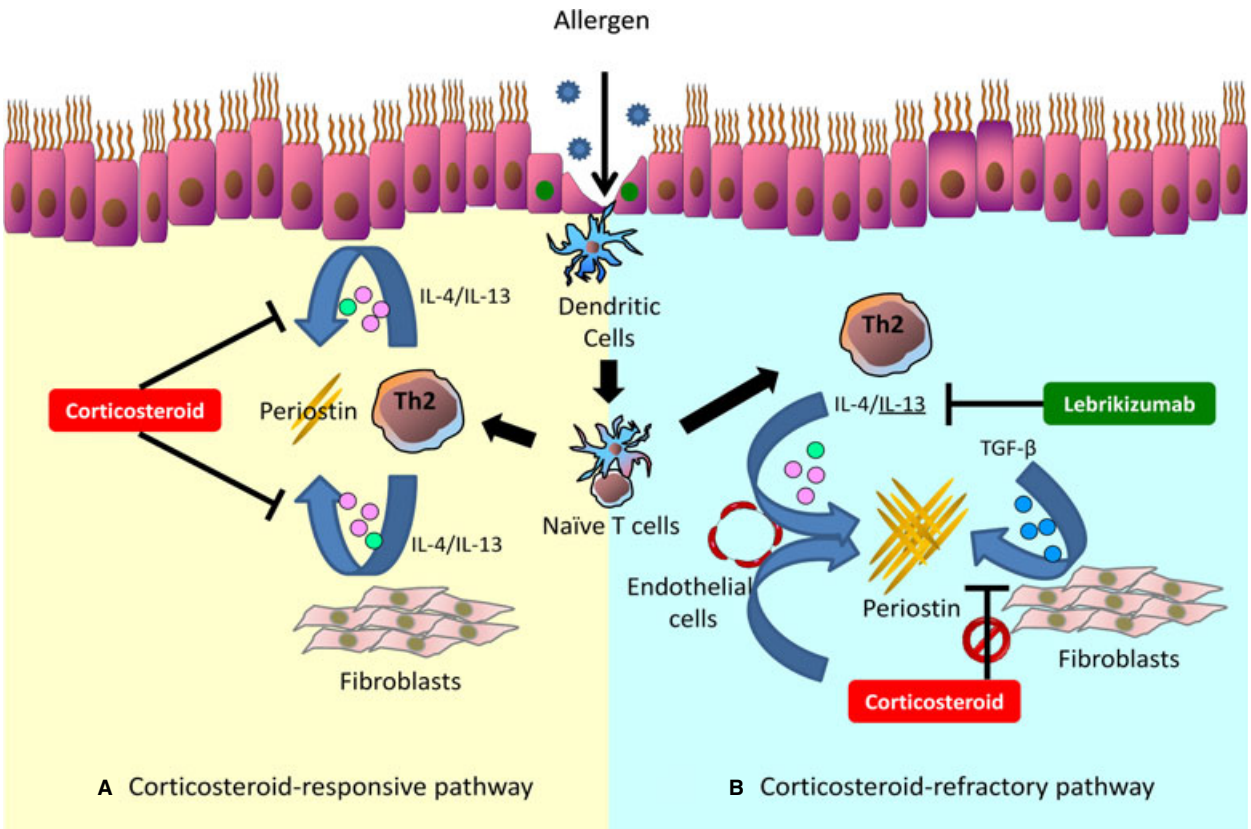


Figure 2 Postulated roles of corticosteroids in the production of periostin in subepithelial tissues. (A) Th2-cytokine-induced periostin production by epithelial cells (2) or fibroblasts (Fig. 1) is effectively inhibited by corticosteroid treatment. (B) When a Th2 cytokine acts on microvascular endothelial cells in the subepithelial tissues, corticosteroid treatment enhances periostin production, rather than inhibit it. Meanwhile, TGF-β-induced periostin production by fibroblasts is not inhibited by corticosteroid treatment. Targeted therapy directed at IL-13, a molecule upstream of periostin, would appear to make sense for severe asthma accompanied by overproduction of periostin despite inhaled corticosteroid (ICS) treatment.

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type-dependent effects of corticosteroids on periostin production may lead to novel therapeutic approaches to corticosteroid-refractory asthma and atopic dermatitis.

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Author contributions

T.S. and K.F. designed the research, performed all the experiments, analyzed the data, and wrote the manuscript. F.K. and H.S. critically revised the manuscripts. K.M. critically revised the manuscripts and received grant support. A.M. designed the research, received grant support, provided overall supervision, analyzed the data, and edited the manuscript.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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