

Activin and transforming growth factor- β signaling pathways are activated after allergen challenge in mild asthma

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Background: Both transforming growth factor (TGF)- β_1 and activin-A have been implicated in airway remodeling in asthma, but the modulation of their specific signaling pathways after disease activation remains undefined.

Objective: To define the expression kinetics of TGF- β_1 , activin-A ligands, and follistatin (a natural activin inhibitor), their type I and type II receptors (activin-like kinase[ALK]-1, ALK-5, ALK-4, T β RII, and ActRIIA/RIIB) and activation of signaling (via phosphorylated (p) Smad2), in the asthmatic airway after allergen challenge.

Methods: Immunohistochemistry was performed on bronchial biopsies from 15 mild atopic patients with asthma (median age, 25 years; median FEV₁% predicted, 97%) at baseline and 24 hours after allergen inhalation. Functional effects of activin-A were evaluated by using cultured normal human bronchial epithelial (NHBE) cells.

Results: pSmad2⁺ epithelial cells increased at 24 hours ($P = .03$), and pSmad2 was detected in submucosal cells. No modulation of activin-A, follistatin, or TGF- β_1 expression was demonstrated. Activin receptor⁺ cells increased after allergen challenge: ALK-4 in epithelium ($P = .04$) and submucosa ($P = .04$), and ActRIIA in epithelium ($P = .01$). The TGF- β receptor ALK-5 expression was minimal in the submucosa at baseline and after challenge and was downregulated in the epithelium after challenge ($P = .02$), whereas ALK-1 and T β RII expression in the submucosa increased after allergen challenge ($P = .03$ and $P = .004$, respectively). ALK-1 and ALK-4 expression by T cells was increased after allergen challenge. Activin-A induced NHBE cell proliferation, was produced by NHBE cells in response to TNF- α , and downregulated TNF- α and IL-13-induced chemokine production by NHBE cells.

Conclusion: Both TGF- β and activin signaling pathways are activated on allergen provocation in asthma. Activin-A may contribute to resolution of inflammation. (J Allergy Clin Immunol 2009;124:454-62.)

Key words: Asthma, activin-A, TGF- β_1

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Abbreviations used

ALK:	Activin-like kinase
BMP:	Bone morphogenetic protein
DAB:	3, 3'-Diaminobenzidine
MCP:	Monocyte chemoattractant protein
NHBE:	Normal human bronchial epithelial
pSmad:	Phosphorylated Smad
TGF:	Transforming growth factor
UK:	United Kingdom

TGF- β_1 and activin-A are members of the TGF- β superfamily of growth factors. Generally TGF- β_1 is a potent inhibitor of inflammation while an activator of tissue fibrosis.¹ Although the exact function of activin-A remains uncertain, it is likely that activin-A also functions to modulate inflammatory responses² while activating tissue repair programs.³ Activin-A is rapidly induced in T_H2 cells on T-cell activation, suggesting that activin-A may also have functions as a T_H2 immunomodulatory cytokine.^{4,5}

TGF- β ligands are present in an inactive state bound to extracellular matrix and as intracellular stores^{6,7}; thus, analysis of signaling pathway components is required to detect functional activity of these ligands. Activated ligand binds to and signals through a serine-threonine kinase-specific type II receptor. TGF- β_1 signaling is via T β RII, whereas activin signaling is predominantly via ActRIIA and ActRIIB. Ligand binding to the type II receptor allows it to complex with and phosphorylate the type I receptor (termed *activin-like kinase* [ALK]), leading to downstream signaling.⁸ The predominant type I receptor for TGF- β_1 is ALK-5, but this cytokine can also bind the more selectively expressed receptor ALK-1. Activins signal through ALK-4. Downstream signaling is via phosphorylation of receptor-regulated Smads that translocate to the nucleus to initiate gene transcription. ALK-5 and ALK-4 signaling is via either phosphorylated (p) Smad2 or Smad3. ALK-1 signaling is via pSmad1/5. Strict regulation of signaling activity is achieved through the induction of inhibitory Smad7, which acts on the type I receptor, leading to receptor degradation. Activins are further regulated by a potent physiological inhibitor, follistatin.⁹

Our group and others have previously shown rapid increases in pSmad2 together with eosinophil-derived TGF- β_1 after allergen provocation in the asthmatic airway.^{10,11} We have also demonstrated rapid induction of airway remodeling and inflammation at 24 hours postallergen challenge.^{10,12} However, a downregulation of the key TGF- β_1 type I receptor, ALK-5, in asthma compared with the normal airway has been previously detected.¹³ In

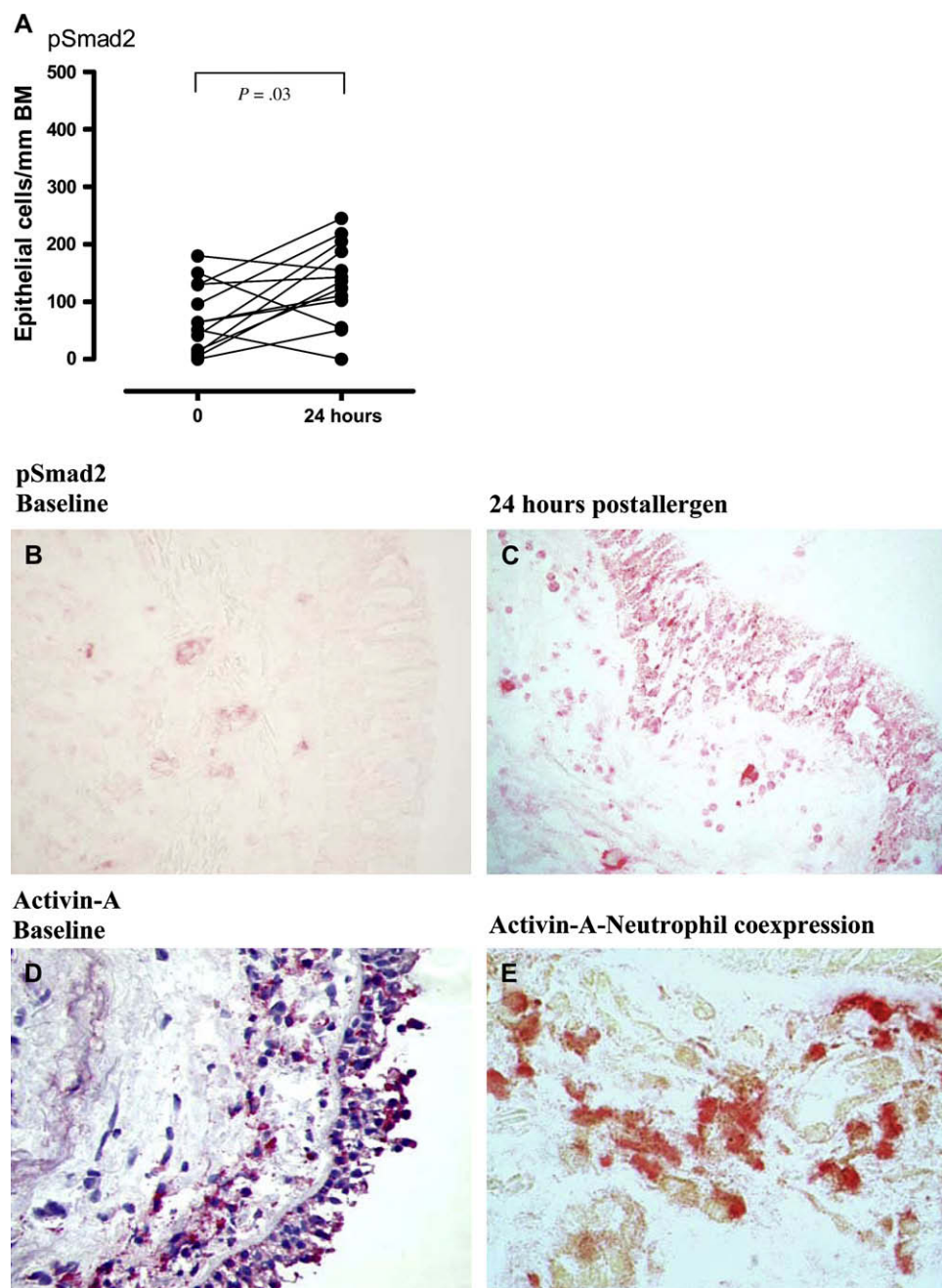


FIG 1. pSmad2 expression at baseline and postallergen. **A**, pSmad2 expression in airway epithelium in response to allergen challenge at the 24-hour time point is summarized. **B and C**, Representative photomicrographs from a paired volunteer at baseline and 24 hours postallergen ($\times 20$ magnification). **D**, Representative photomicrograph of activin-A expression in the airway ($\times 40$). **E**, Colocalization (brown via DAB) to elastase⁺ neutrophils (Fast Red; $\times 100$). BM, Basement membrane.

addition, low levels of ALK-5 expression are present in a murine model of allergen-induced airway injury¹⁴ and lung injury–fibrosis.¹⁵ These data suggest that other TGF- β receptors and/or other cytokines may be involved in chronic allergic airway inflammation and remodeling in asthma. Activin-A has been implicated in airway inflammation in mouse models of allergen challenge,¹⁴ was elevated in serum from symptomatic patients with asthma, and was detected in peripheral blood T_H2 cells from patients with asthma.⁴ We therefore hypothesized that rapid expression of pSmad2 in the airways after allergen challenge in asthma

may be related to activation of activin-A signaling. Here, we examined the time course of activation of TGF- β and activin-A signaling and receptor modulation at baseline and 24 hours after allergen challenge in mild asthma.

METHODS

Volunteer details and study design

The study was approved by the Royal Brompton and Harefield Hospital Ethics Committee, and volunteers gave written informed consent. Fifteen volunteers with a history of atopic asthma together with either a 15%

TABLE I. Summary of ligand and signaling component expression

Protein	Epithelium		Submucosa	
	Baseline	24 Hours	Baseline	24 Hours
TGF- β_1	197.3 (67-236)	240 (174.4-272)	40 (26.7-60)	44.80 (21.35-72)
Activin-A	132 (80-170.7)	114 (99.20-153)	60 (32-112)	83 (32-147.2)
Follistatin	108 (0-156)	121.6 (48-192)	Absent or minimal	Absent or minimal
ALK-5	225.6 (132.2-344.9)	154.7* (64.70-210.2)	Absent or minimal	Absent or minimal
ALK-1	116.4 (77.85-177.5)	169 (64.35-356)	0 (0-9.35)	18.30* (0-65.35)
ALK-4	178.7 (152-240)	236* (224-310)	10.70 (1.35-36.80)	59.50* (6.4-146)
T β RII	352 (304-400)	360 (320-440)	115.2 (32-148)	213.3* (144-260)
ActRIIA	0 (0-67.2)	99* (0-169.6)	4 (0-24)	12 (0-39.2)
ActRIIB	131.2 (48-166.4)	144 (96-196)	Absent or minimal	Absent or minimal
pSmad2	64 (14-130.5)	136* (78.65-196.2)	4 (0-9.6)	17.6 (2.3-69.3)

Epithelial expression (median $\pm$ interquartile range) is given as the number of cells per unit length of basement membrane (cells/mm basement membrane). Expression in the submucosa is expressed as cells/mm².

* $P < .05$.

increase in FEV₁ to β_2 agonist or methacholine PC₂₀ $\leq$ 8 mg/mL were recruited. The median age was 25 years (range, 19-46 years), with a FEV₁ % predicted of 97% (range, 75.4% to 125.7%) at study entry with a methacholine PC₂₀ of 2.1 mg/mL (1.2-3.6 mg/mL; geometric mean $\pm$ 95% CI). All subjects demonstrated positive skin prick tests (wheal size $\geq$ 3 mm) to 1 or more of the aeroallergens house dust mite, cat dander, or grass (ALK, Hørsholm, Denmark). Volunteers sensitive to pollens were studied outside of the season. Volunteers were controlled with only rescue β_2 -agonists at the time of study and had no clinical features of infection for at least 4 weeks before starting the study and none throughout the study period. The study design has been described previously.¹² Briefly, bronchial biopsies obtained at baseline and then 24 hours postallergen challenge were evaluated. All volunteers were nonsmokers. All bronchoscopies were performed between 8:30 and 9:00 A.M.

Immunohistochemistry

Tissue processing and immunostaining was performed as previously described,^{16,17} as was the alkaline phosphatase-antialkaline phosphatase method¹⁶ to identify specific binding of antibodies to cells. The alkaline phosphatase-antialkaline phosphatase reaction was visualized by using appropriate Vectastain-ABC-AP kits (Vector Laboratories, Peterborough, UK) and the Fast Red chromogen. A chicken polyclonal antibody against TGF- β_1 and a polyclonal goat antibody against human activin-A were used (R&D Systems, Abingdon, United Kingdom [UK]). A polyclonal goat antibody against follistatin was used (R&D Systems). Inflammatory cell colocalization of activin-A was performed by using a standard double-staining technique. Briefly, activin-A expression was localized by using 3, 3'-diaminobenzidine (DAB) chromogen that produces a brown end product, whereas inflammatory cell markers were identified using Fast Red as previously described.¹² The antibodies directed against the type I and type II receptors and Smads were a kind gift from Prof P. Sideras, Biomedical Research Foundation of the Academy of Athens, Greece. Briefly, polyclonal antibodies were raised in rabbits against synthetic polypeptides and tested for specificity by immunoprecipitation and Western blotting as previously described.^{14,18} These antibodies have been previously validated in human tissue.¹⁹ Double-staining for ALK-1 (Autogen Bioclear, Wiltshire, UK), ALK-4 (GeneTex, Irvine, Calif) and T β RII (R&D Systems) with CD3 (Dako, Cambridge, UK) was performed as previously described.¹⁶ Incubation of tissue sections with an irrelevant species IgG antibody served as a negative control. Cells counts were done in a blind fashion by an independent observer by using an Olympus BH-2 Microscope (Olympus Corp, Lake Success, NY) as previously described.¹⁰

Epithelial cell culture and stimulation

The primary cultured normal human bronchial epithelial (NHBE) cells (Lonza Rockland Inc, Rockland, Me) were seeded in 6-well plastic plates (Sigma-Aldrich, Gillingham, UK) previously coated with 2.5 mg/mL collagen type I (Sigma-Aldrich) in 0.016 mmol/L acetic acid. Cells were grown at 37°C

in a humidified 5% CO₂ atmosphere in bronchial epithelium growth medium supplemented with a bullet kit containing 0.5 ng/mL recombinant human epidermal growth factor, 500 ng/mL hydrocortisone, 0.005 mg/mL insulin, 0.035 mg/mL bovine pituitary extract, 500 nmol/L ethanolamine, 500 nmol/L phosphoethanolamine, 0.01 mg/mL transferrin, 6.5 ng/mL 3,3,5-triiodothyronine, 500 ng/mL adrenaline, and 0.1 ng/mL retinoic acid (Lonza Rockland Inc). Once they reached 80% confluence, epithelial cells were used for experiments. Activin-A, follistatin, IL-13, and TNF- α were all from R&D Systems.

The effect of activin-A on NHBE cell proliferation was determined by using the ViaLight Cell proliferation BioAssay Kit (Lonza Rockland Inc) according to the manufacturer's instructions after 24 hours of stimulation. The concentrations of IL-6, CXCL8/IL-8, IL-13, and CCL5/RANTES were assessed by ELISA (Peptrotech EC, London, UK), and activin-A was measured by activin-A Duoset ELISA (R&D Systems). A Human Chemokine Ten-Plex Antibody Bead Kit (Invitrogen, Carlsbad, Calif) was used to detect the level of CCL11/eotaxin, CXCL1/growth related oncogene (GRO)- α , CXCL10/inducible protein (IP)-10, CXCL9/monokine induced by gamma-interferon (MIG), CCL2/monocyte chemoattractant protein [MCP]-1, CCL8/MCP-2, CCL7/MCP-3, CCL3/macrophage inflammatory protein-1 α , CCL4/ β , and CCL5/RANTES; the plate was analyzed with a Luminex 100TM instrument (Luminex xMAP Technology, Oosterhout, The Netherlands). ELISAs and the Luminex plate were all assessed on supernatants from the 24-hour stimulation time point.

Statistical analysis

Cell counts are presented as the median $\pm$ interquartile range unless otherwise stated. All paired within-subject data were analyzed by using the Wilcoxon signed-rank test. For time course experiments, comparability between the means was assessed by the Friedman test and then the Wilcoxon test as a posttest. Data were analyzed by using Graph Pad Prism Version 4 (Graph Pad Software Inc, San Diego, Calif) or StatView (SAS Institute, Inc, San Francisco, Calif). Significance was accepted as $P < .05$.

RESULTS

Activation of pSmad2 signaling is seen 24 hours after allergen challenge

Allergen challenge was associated with significant increases in the number of pSmad2-positive epithelial cells at 24 hours postallergen challenge ($P = .03$; $n = 13$ volunteers; Fig 1, A-C), suggesting rapid activation of TGF- β and/or activin signaling in response to allergen. Submucosal cells also stained positive for pSmad2 after allergen challenge, although this increase was not significant (Table I).

TGF- β_1 and activin-A were expressed in the airway of patients with mild asthma at baseline. There was no modulation of numbers

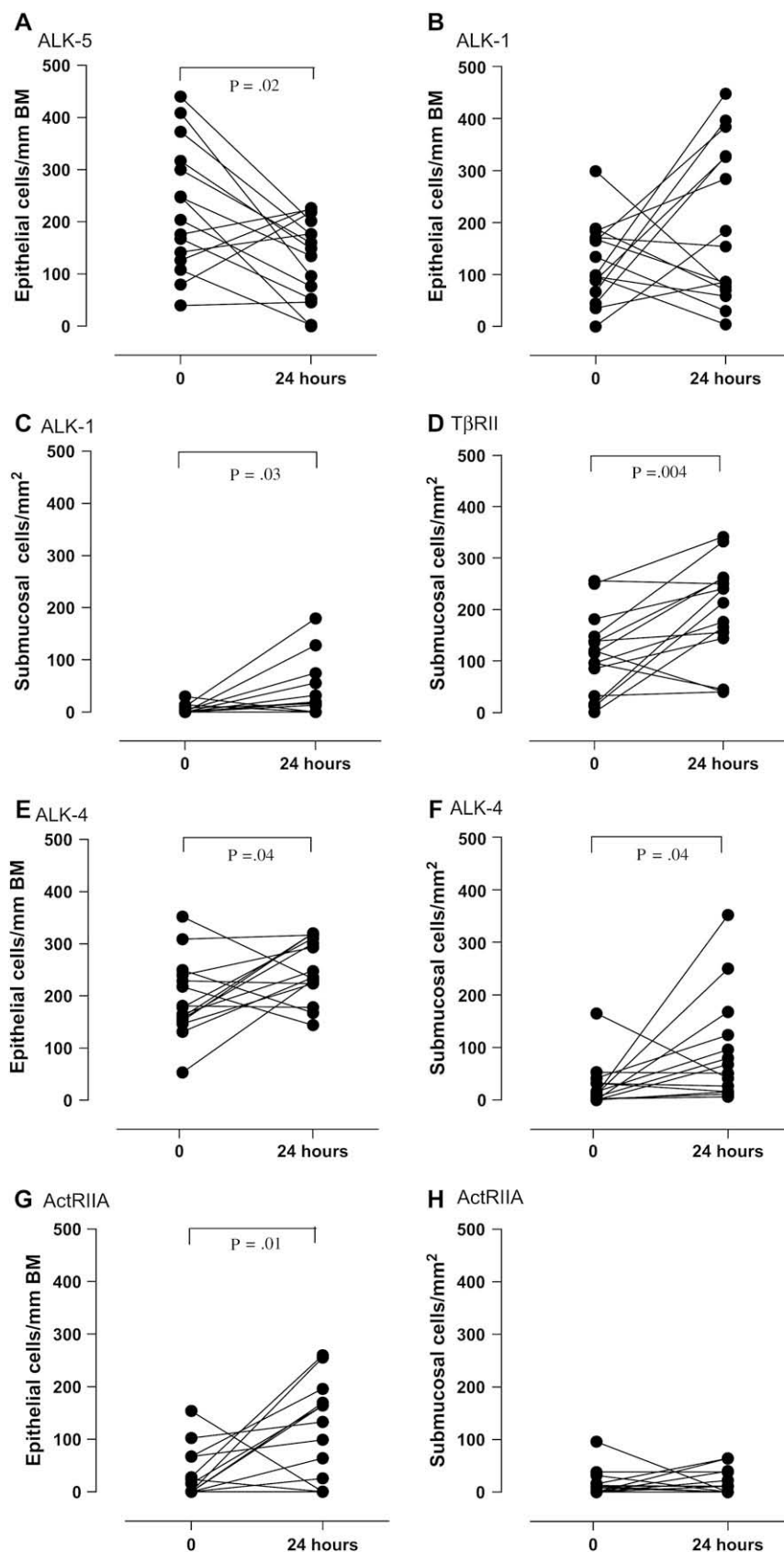


FIG 2. TGF- β and activin receptor modulation after allergen provocation in asthma. Epithelial ALK-5 (**A**) and ALK-1 (**B**) (submucosa, **C**) expression before and after allergen challenge is summarized. Submucosal cell expression of T β RII is also presented (**D**). ALK-4 (**E** and **F**) and ActRIIA (**G** and **H**) expression is summarized. Epithelial cells are expressed as cells/mm BM and submucosal cells as cells per mm². BM, Basement membrane.

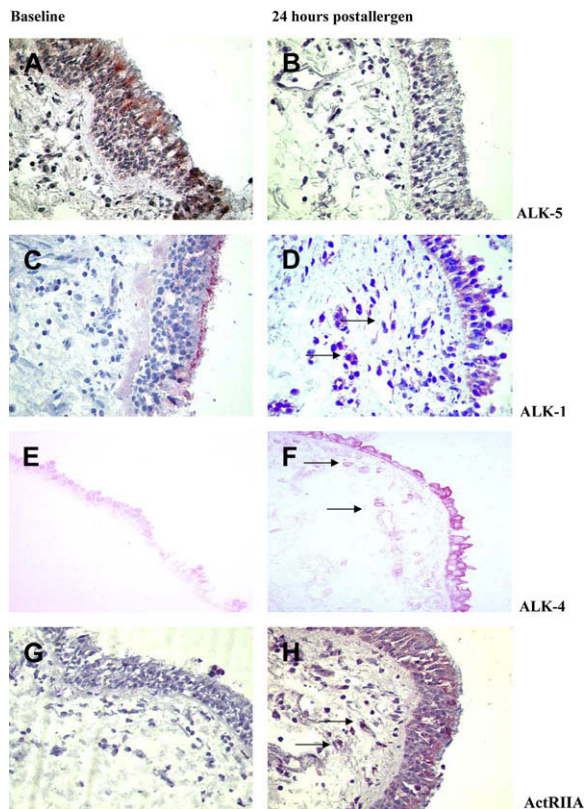


FIG 3. Receptor modulation in response to allergen challenge. Representative photomicrographs of ALK-5 expression (**A and B**), ALK-1 (**C and D**), ALK-4 (**E and F**), and ActRIIA (**G and H**) at baseline and postallergen ($\times 40$ magnification). Submucosal cells expressing ALK-1, ALK-4, and ActRIIA are *arrowed*.

of cells positive for TGF- β_1 , activin-A, or follistatin postallergen challenge in either epithelium or submucosa (**Table I**). Of the activin-A–positive submucosal cells, 51.1% (24.9–59.1) were neutrophils. In addition, at 24 hours, 32.5% (11.7–46.2) of the infiltrating neutrophil population stained for activin-A ($n = 6$). Mast cells (tryptase $^+$), CD4 $^+$ T cells, and macrophages (CD68 $^+$) were also identified as sources of activin-A (data not shown). Representative photomicrographs of mucosal activin-A expression and colocalization to neutrophils are shown (**Fig 1, D and E**).

Allergen inhalation challenge modulates type I and type II receptor expression for TGF- β and activin-A

Because both TGF- β_1 and activin-A signal via pSmad2, and both ligands are expressed in asthma, we examined the effect of allergen challenge on type I and type II receptor expression both for TGF- β_1 (ALK-1, ALK-5, and T β RII) and activin-A (ALK-4 and ActRIIA/ActRIIB).

TGF- β receptors

Allergen challenge was associated with a decrease in the number of epithelial cells expressing ALK-5 at 24 hours ($P = .02$; **Fig 2, A**; **Fig 3, A and B**). Scattered submucosal inflammatory-like cells staining positive for ALK-5 were identified in low numbers only and not in all volunteers. Similarly, ALK-5 expression was not detected in either fibroblastlike cells or airway smooth muscle cells. However, there was increased expression of ALK-1 in

epithelial cells from baseline to 24 hours postallergen challenge (**Table I**; **Fig 2, B**; **Fig 3, C and D**). Furthermore, significantly increased numbers of submucosal cells expressed ALK-1 at 24 hours ($P = .03$; **Fig 2, C**; **Fig 3, C and D**). No modulation of epithelial T β RII expression was found (**Table I**). There were significantly increased numbers of submucosal cells expressing T β RII at the 24-hour time point after allergen challenge ($P = .004$; **Fig 2, D**). ALK-1 was expressed on CD3 $^+$ T cells at baseline, and expression was increased postallergen challenge ($P = .03$; $n = 6$; **Fig 4, A**). After allergen challenge, 71.65% (median) of CD3 $^+$ T cells were ALK-1 $^+$ (58.55–84.70). Both before and after allergen challenge, all CD3 $^+$ T cells identified also stained for T β RII (data not shown).

Activin receptors

At 24 hours after allergen challenge, there were increased numbers of epithelial cells ($P = .04$) (**Fig 2, E**) and submucosal inflammatory-like cells staining for ALK-4 ($P = .04$; **Fig 2, F**). ALK-4 expression was evident in fibroblastlike cells postallergen ($3.2 \text{ cells/mm}^2 \pm 0.35.2 \text{ range}$ vs $11.40 \text{ cells/mm}^2 \pm 4.190 \text{ range}$). Increased numbers of epithelial cells stained for ActRIIA at 24 hours after allergen challenge ($P = .01$; **Fig 2, G**). Representative photomicrographs are given in **Fig 3, E and F**, and **Fig 3, G and H**. There was a nonsignificant trend for increased numbers of submucosal cells staining for ActRIIA postallergen (**Fig 2, H**). No modulation of ActRIIB was demonstrated in either tissue compartment (**Table I**). ALK-4 was expressed on CD3 $^+$ T cells at baseline with rapid modulation of expression postallergen ($P = .03$; $n = 6$; **Fig 4, B and C**). After allergen challenge, 96.5% (median) of CD3 $^+$ T cells were ALK-4 $^+$ (91.89–100).

Activin-A induces proliferation of and inhibits cytokine-induced chemokine production by primary human bronchial epithelial cells

Normal human bronchial epithelial cells were stimulated with increasing doses of activin-A for 6, 24, and 48 hours. A dose-dependent increase in NHBE cell proliferation was observed at each time point, reaching significance at 10 and 25 ng/mL (**Fig 5, A**). Activin did not induce release of IL-6, CXCL8/IL-8, IL-13, CCL11/eotaxin, CXCL1/GRO- α , CXCL10/IP-10, CXCL9/MIG, CCL2/MCP-1, CCL8/MCP-2, CCL7/MCP-3, CCL3/macrophage inflammatory protein-1 α , CCL4/ β , or CCL5/RANTES from NHBE (data not shown). TNF- α increased the release of activin-A by NHBE cells, which also released activin-A without stimulation (**Fig 5, B**). Furthermore, the activin inhibitor follistatin augmented IL-13 induction of CXCL8/IL-8 by NHBE (**Fig 5, C**). In addition, although at the concentrations tested, TNF- α and IL-13 did not induce release of CXCL10/IP-10 or CCL2/MCP-1 from NHBE, blockade of activin by follistatin induced significant production of both chemokines by IL-13 or TNF- α –stimulated NHBE (**Fig 5, D and E**, and data not shown), suggesting that activin acts to inhibit cytokine-induced chemokine production by bronchial epithelial cells.

DISCUSSION

This study suggests that rapid activation of pSmad2 in response to allergen challenge in asthma may result from signaling by both activins and TGF- β . We report rapid modulation of selected

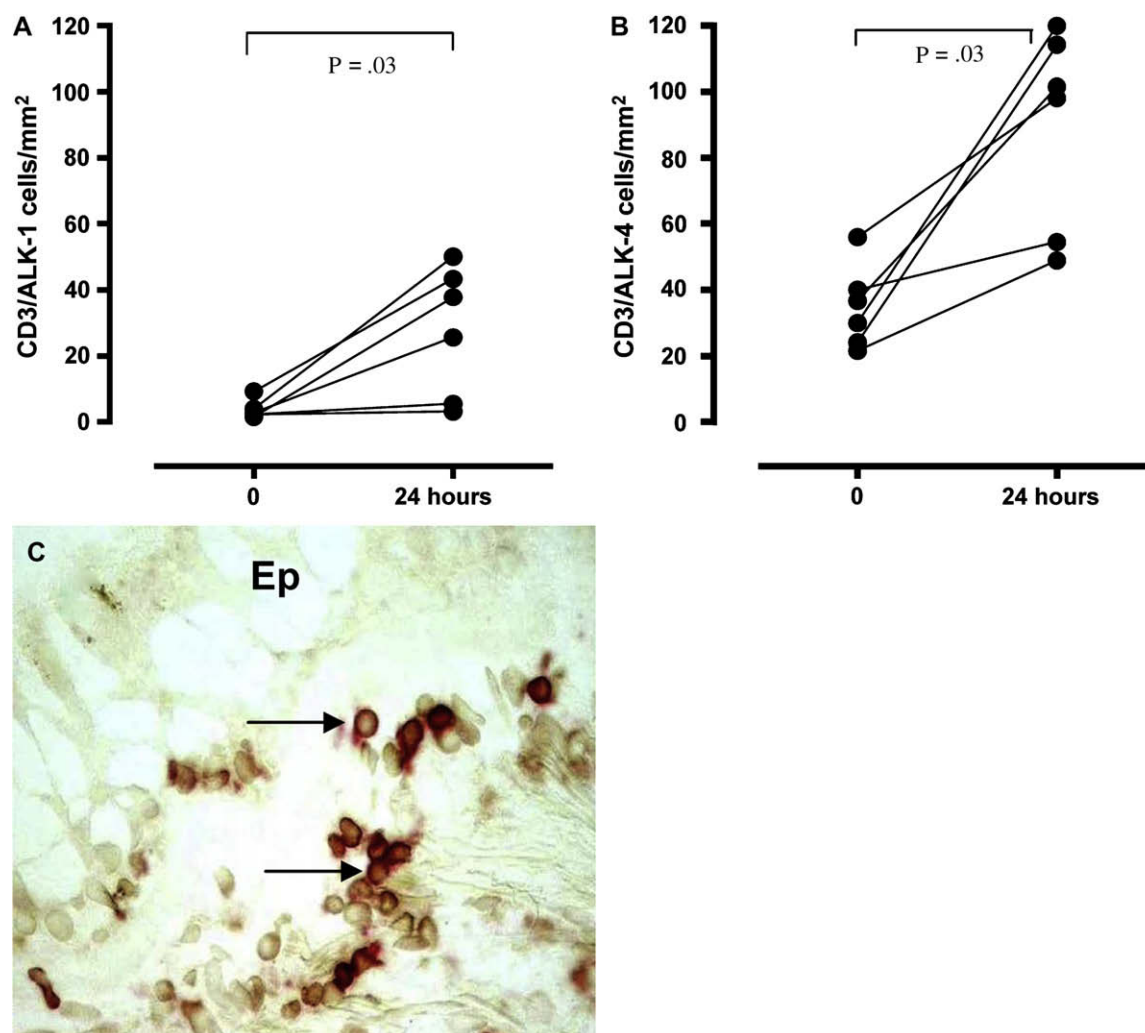


FIG 4. ALK-1 and ALK-4 modulation of expression CD3⁺ T cells postallergen. Numbers of T cells expressing receptors for TGF- β (ALK-1, **A**) and activin-A (ALK-4, **B**) were enumerated before and after allergen challenge by double-staining for CD3 and ALK-1 or ALK-4. Counts are expressed as cells per mm² biopsy. **C**, A representative photomicrograph for double-staining for CD3 (red) and ALK-4 (DAB). Double-stained cells are darker red-brown. The epithelium is indicated as Ep to orientate the section. N = 6.

ligand-specific receptor expression. In particular ALK-5, the type I receptor implicated to date in TGF- β signaling was downregulated in airway epithelium with absent or decreased expression in the submucosa, whereas we detected ALK-1 expression by airway epithelium and submucosal cells with increases after allergen challenge, raising the possibility that TGF- β may also signal via ALK-1 in the asthmatic airway. ALK-4, the only activin type I receptor, was expressed at baseline and further upregulated in response to allergen challenge, suggesting that activin-mediated signaling pathways have important roles in the airway response to allergen-induced airway inflammation and remodeling events in asthma. Activin-A induced proliferation of bronchial epithelial cells in culture and inhibited cytokine-induced chemokine release by these cells.

Neither TGF- β nor activin-A ligand expression was modulated in response to disease activation in our study. Torrego et al¹¹ have previously reported an increase in TGF- β (but not TGF- β or TGF- β expression in the airway after allergen challenge), whereas Rosendahl et al¹⁴ reported an increase in mRNA for TGF- β and activin-A in lungs from mice sensitized and challenged with ovalbumin, but no changes in mRNA for

TGF- β or TGF- β . However, because both TGF- β and activin-A are stored in tissues in inactive forms and immunohistochemistry and *in situ* hybridization cannot distinguish inactive forms from activated ligands, we suggest that detection of pSmad2 may indicate activation of both TGF- β and activin pathways after allergen challenge in asthma.

Here we identified neutrophils as a source of activin-A in the asthmatic airway after allergen challenge. Neutrophils have already been identified as an important source of TGF- β in asthma²⁰ and thus may have a role in tissue remodeling. The exact contribution of neutrophil-derived activin-A to asthma pathogenesis will need further focus.

The rapid and consistent downregulation in the expression pattern of epithelial ALK-5 at 24 hours after allergen exposure raises the possibility that there may be a regulatory mechanism in place to attenuate the cellular response to TGF- β . This observation is in keeping with data from an allergen-induced mouse model of airway injury¹⁴ and a rat model of bleomycin-induced lung fibrosis,¹⁵ both of which demonstrated reduced expression of ALK-5 with activation of fibrosis. ALK-5 expression was not

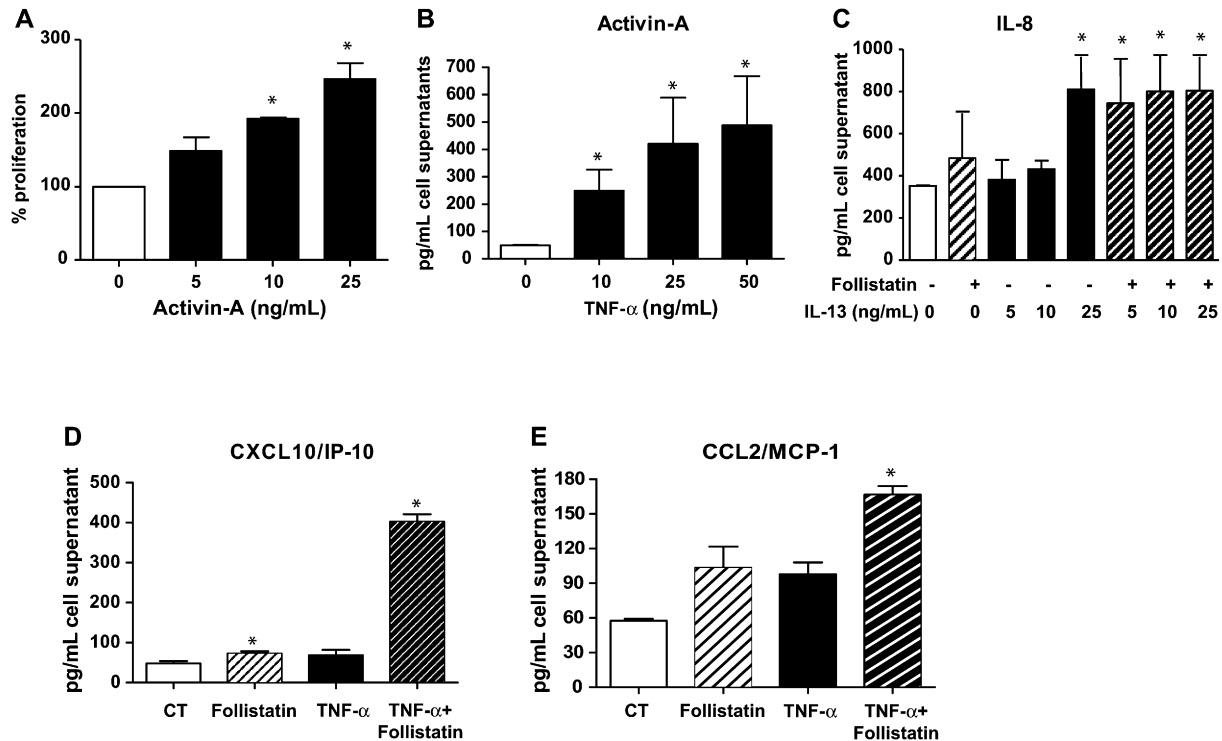


FIG 5. Effect of activin-A on human bronchial epithelial cells. **A**, Activin-A caused dose-dependent proliferation of NHBE cells in culture. Data are for 24 hours of culture and are means and SEs for 5 separate experiments. **B**, TNF- α induced dose-dependent release of activin-A from NHBE cells (24-hour time point; means and SEs; $n = 5$). **C**, IL-13 at 10 ng/mL induced dose-dependent release of IL-8 from NHBE cells, and this was significantly increased in the presence of 100 ng follistatin (24-hour time point; $n = 5$). **D and E**, TNF- α alone did not induce release of CXCL10/IP-10 or CCL2/MCP-1 from NHBE cells, but this was significantly induced in the presence of the activin-inhibitor follistatin at 100 ng/mL ($n = 5$). * $P < .05$ for comparison with medium-alone values. CT, Control.

detected on submucosal inflammatory-like cells at any time in the patients with mild asthma studied here. However, decreased ALK-5 expression has been documented in the asthmatic airway in more symptomatic subjects previously.¹³ Inflammatory cell expression of ALK-5 is related to the state of cell differentiation and activation, as has been demonstrated in inactive monocytes that express a relatively high proportion of ALK-5 early on, but with cell activation there is downregulation of ALK-5 with concomitant loss of functional responses to TGF- β ligand.²¹

ALK-1 expression was increased after allergen challenge in epithelium and particularly submucosal cells. Unidentified stromal cells of nasal tissue have been shown to express ALK-1,²² and a mouse model of allergen-induced airway injury demonstrates ALK-1 expression in submucosal infiltrating cells, fibroblasts, epithelium, and vascular structures.¹⁴

The functional consequence of ALK-1 expression in the context of airway inflammation and remodeling in asthma remains to be determined. In endothelial cells, at least ALK-1 activation leads to cell proliferation and migration, whereas ALK-5 signaling antagonizes such responses.^{23,24} Although we appreciate that our data are derived from an immunohistochemical approach which is semiquantitative at best and that several pathways will interact with the TGF- β signaling cascade, it is still important to consider the possibility that the trend toward increased ALK-1 expression alongside decreased or absent ALK-5 expression observed here may reflect downregulation of ALK-5-mediated signaling programs while antagonistic ALK-

1-mediated signaling programs are activated. ALK-1 signals through the Smad1/5 pathway, and our recent work demonstrating increased allergen-induced signaling of pSmad1/5 expression would thus also support ALK-1-mediated signalling.²⁵ Several bone morphogenetic proteins (BMPs) such as BMP-7 are antifibrotic, and it is therefore possible that ALK-1 induction is an attempt at regulating the airway-injury response.²⁶ It will be important to understand how TGF- β 1 and the BMP-activated ALK-1 interact to determine functional cellular outcomes.

In contrast with ALK-5, ALK-4 expression increased throughout the epithelium and submucosal cells after allergen challenge. Furthermore, rapid upregulation of ActRIIA was detected in the epithelium after challenge with increased numbers of submucosal cells also expressing ActRIIA. Given the absence of ALK-5 expression in the airway submucosa in our study and others,¹³ these findings may suggest that activin-A may be an important contributor to airway responses to allergen challenge. To support this in animal models of lung fibrosis, the activin antagonist follistatin abolishes fibrosis even in the presence of TGF- β 1,²⁷ and fibroblasts rapidly upregulate ALK-4 expression.¹⁴ Here, we detected ALK-4 expression by fibroblastlike cells but did not see any upregulation of follistatin after allergen challenge of patients with asthma, suggesting that activin-A may act unopposed to activate airway fibroblasts. These findings support and extend those of Karagiannidis et al,⁴ who showed increased activin-A in serum from symptomatic patients with asthma and activation of airway fibroblasts *in vitro* by activin-A.

The observation of increased ALK-4 expression and pSmad2 activation in airway epithelium after allergen challenge in asthma led us to examine the effects of activin-A on primary human airway epithelial cells in culture. Activin-A induced proliferation but not cytokine or chemokine release by NHBE cells. Furthermore, our data using the natural activin inhibitor, follistatin, raise the possibility that activin may act as an inhibitor of cytokine-induced proinflammatory chemokine release from the airway epithelium. These findings lead us to postulate a role for activin signaling in repair and resolution of inflammation after allergen challenge in asthma. Interestingly, rhinovirus infection also induces activin-A release from bronchial epithelial cells, and it will be of interest to determine whether this cytokine has a role in resolution of virus-induced airway inflammation.²⁸ TGF- β_1 is also reported to inhibit cytokine-induced chemokine production from epithelial cells²⁹ and increases mucin production.³⁰

Our demonstration of the expression of ALK-1 and ALK-4 on CD3⁺ T cells and modulation of expression in response to allergen-provocation of asthma suggests that both TGF- β_1 and activin-A may act in resolution of T cell-mediated airway inflammation, because both cytokines can suppress effector T-cell function.^{31,32} Activin-A has recently been reported to synergize with TGF- β_1 for expansion of regulatory T cells.³³ The increase in ALK-4 after allergen challenge may thus represent an activin-A-mediated inherent protective mechanism for resolution of inflammation. In addition, TGF- β_1 , together with IL-6 acts in the differentiation of T_H17 cells, and this may be relevant to a possible role for such cells in chronic asthma.³⁴ Further studies will be required to explore these areas.

In conclusion, allergen provocation of asthma leads to rapid activation of TGF- β and activin signaling pathways, whereas receptor expression and our studies of airway epithelial cell function suggest a role for activin-A in resolution of inflammation and initiation of airway remodeling after allergen challenge. Alternate TGF- β_1 pathways via ALK-1 rather than ALK-5 may also be operative. Further interventional approaches will be required to dissect these pathways *in vivo*, but it is clear that targeting TGF- β superfamily signaling in asthma will be ineffective unless the integrated and interactive signaling pathways that are in operation are considered as a whole.

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Key messages

Activin-A receptor upregulation alongside downregulation of expression of TGF- β_1 receptor ALK-5 after allergen challenge, together with activin-A-induced bronchial epithelial cell proliferation and evidence that activin-A inhibits chemokine release from epithelium, suggests that activin-A may have a role in allergen-induced remodeling and resolution of inflammation in asthma.

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