

# IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH<sub>2</sub>-terminal kinase-dependent pathway

Shu Hashimoto, MD, Yasuhiro Gon, MD, Ikuko Takeshita, BS,  
Shuichiro Maruoka, MD, and Takashi Horie, MD *Tokyo, Japan*

**Background:** Myofibroblasts play a role in the airway remodeling response of bronchial asthma. IL-4 and IL-13 are possibly involved in the airway remodeling response by inducing extracellular matrix production by fibroblasts. However, the roles of these cytokines in inducing the phenotypic modulation of human lung fibroblasts (HLFs) to myofibroblasts and the intracellular signal have not been determined.

**Objective:** We examined the effect of IL-4 and IL-13 on inducing the phenotypic modulation of HLFs to myofibroblasts characterized by  $\alpha$ -smooth muscle actin and examined the role of the mitogen-activated protein (MAP) kinase superfamily in inducing the myofibroblastic phenotype of the HLF to clarify these issues.

**Methods:** Phosphorylation and activities of c-Jun NH<sub>2</sub>-terminal kinase (JNK), p38 MAP kinase, and extracellular signal-regulated kinase (Erk) were examined by using Western blotting and in vitro kinase assay. Expression of  $\alpha$ -smooth muscle actin in IL-4- and IL-13-stimulated HLFs was analyzed by means of Western blotting.

**Results:** The results showed that (1) IL-4 and IL-13 increased  $\alpha$ -smooth muscle actin expression in a dose- and time-dependent manner; (2) IL-4 and IL-13 induced increases in JNK and Erk phosphorylation and activity but not p38 MAP kinase activity; (3) CEP-1347 and PD 98059 attenuated IL-4- and IL-13-induced JNK and Erk activity, respectively; and (4) CEP-1347, but not PD 98059, attenuated IL-4- and IL-13-induced  $\alpha$ -smooth muscle actin expression.

**Conclusion:** These results indicate that IL-4 and IL-13 are capable of inducing the phenotypic modulation of HLFs to myofibroblasts, and JNK, but not p38 MAP kinase and Erk, regulates IL-4- and IL-13-induced phenotypic modulation of HLFs to myofibroblasts. (*J Allergy Clin Immunol* 2001;107:1001-8.)

**Key words:** IL-4, IL-13, human lung fibroblast, myofibroblast, mitogen-activated protein kinase, c-Jun NH<sub>2</sub>-terminal kinase

## Abbreviations used

DMSO: Dimethylsulfoxide  
Erk: Extracellular signal-regulated kinase  
HLF: Human lung fibroblast  
JNK: c-Jun NH<sub>2</sub>-terminal kinase  
MAP: Mitogen-activated protein  
TGF- $\beta$ <sub>1</sub>: Transforming growth factor  $\beta$ <sub>1</sub>

Airway remodeling has been documented to be a prominent feature of the bronchial wall of patients with chronic severe asthma.<sup>1-3</sup> Many elements contribute to the airway remodeling response (ie, basement membrane thickening associated with subepithelial extracellular matrix deposition and subepithelial fibrosis).<sup>1-3</sup> Subepithelial myofibroblast hyperplasia has been demonstrated in patients with asthma, as well as in animal models of asthma.<sup>4-6</sup> Myofibroblasts are major producers of extracellular matrix proteins.<sup>7-9</sup> They may serve a similar role in asthma because the number of myofibroblasts present in the submucosa correlates with subepithelial collagen deposition.<sup>4,7</sup> Thus myofibroblasts may play a role in the airway remodeling response of asthma, and therefore it is important to clarify the mechanism in the phenotypic modulation of lung fibroblasts to myofibroblasts in bronchial asthma. It is well known that T<sub>H</sub>2 cytokines play a key role in the production of allergic inflammation in asthma.<sup>10</sup> Among T<sub>H</sub>2 cytokines, IL-4 receptor and IL-13 receptor are expressed in fibroblasts,<sup>11,12</sup> and IL-4 and IL-13 elicit various cell functions of fibroblasts, including cytokine and adhesion molecule expression and extracellular matrix protein synthesis.<sup>13-17</sup> These observations indicate that these cytokines play a role in the airway remodeling response of asthma; however, the role of IL-4 and IL-13 in inducing the phenotypic modulation of lung fibroblasts to myofibroblasts has not been determined.

Many extracellular stimuli elicit specific biologic responses through activation of mitogen-activated protein (MAP) kinase cascades.<sup>18</sup> The mammalian MAP kinase superfamily has been molecularly characterized and consists of extracellular signal-regulated kinase (Erk), p38 MAP kinase, and c-Jun NH<sub>2</sub>-terminal kinase (JNK). p38 MAP kinase and JNK are activated by environmental stresses and play an important role in apoptosis and cytokine expression,<sup>19-21</sup> whereas Erk is activated by mitogenic stimuli and plays a central role in cell proliferation

From the First Department of Internal Medicine Nihon University School of Medicine, Tokyo.

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Reprint requests: Shu Hashimoto, MD, First Department of Internal Medicine Nihon University School of Medicine, 30-1 Oyaguchikamimachi, Itabashi-ku, Tokyo 173-8610, Japan.

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and differentiation.<sup>22</sup> However, recent studies have suggested that Erk and JNK also play important roles in the signal cascades leading to the induction of various inflammatory mediators.<sup>23,24</sup> Thus the MAP kinase superfamily regulates a variety of cellular functions. We have previously shown that JNK regulates the phenotypic modulation of human lung fibroblasts (HLFs) to myofibroblasts in transforming growth factor  $\beta_1$  (TGF- $\beta_1$ )-stimulated HLFs.<sup>25</sup> However, the role of the MAP kinase superfamily in the phenotypic modulation of HLFs to myofibroblasts in IL-4- and IL-13-stimulated HLFs has not been determined.

Therefore in this study we examined whether IL-4 and IL-13 could induce the phenotypic modulation of HLFs to myofibroblasts characterized by  $\alpha$ -smooth muscle actin expression and analyzed the role of JNK, p38 MAP kinase, and Erk in inducing the myofibroblastic phenotype. To this end, we examined  $\alpha$ -smooth muscle actin expression and JNK, p38 MAP kinase, and Erk phosphorylation and activity, as well as the effect of CEP-1347 as the specific inhibitor of the JNK signaling pathway,<sup>26</sup> SB 203580 as the specific inhibitor of p38 MAP kinase,<sup>27</sup> and PD 98059 as the specific inhibitor of MAP kinase kinase 1, which is an upstream of Erk,<sup>28</sup> in the induction of  $\alpha$ -smooth muscle actin expression in IL-4- and IL-13-stimulated HLFs.

## METHODS

### Reagents

SB 203580 and PD 98059 were obtained from Carbiochem-Novabiochem Corporation (La Jolla, Calif) and New England Biolabs, Inc (Beverly, Mass). Anti-TGF- $\beta_1$  antibody, anti-IL-4 antibody, and anti-IL-13 antibody were obtained from Genzyme TECHNE (Minneapolis, Minn). CEP-1347 was kindly provided by Cephalon Incorporated (West Chester, Pa). SB 203580, PD 98059, and CEP-1347 were dissolved in dimethylsulfoxide (DMSO).

### Cell cultures

HLFs were obtained from Clonetics (San Diego, Calif). In the preliminary experiments we immunocytochemically stained HLFs with a specific antibody to  $\alpha$ -smooth muscle actin (ZYMED Labs Inc, San Francisco, Calif) followed by FITC-conjugated anti-mouse IgG. The cells were viewed with an Olympus confocal laser microscope (Olympus Kogyo Corp Ltd, Tokyo, Japan) to examine the homogeneity of HLFs with regard to  $\alpha$ -smooth muscle actin expression. Unstimulated HLFs were homogeneously and very weakly stained, indicating that the HLFs used in this study were homogeneous with regard to  $\alpha$ -smooth muscle actin expression. HLFs were placed onto collagen-coated tissue culture plates (Iwaki, Tokyo, Japan) for Western blot analysis and in vitro kinase and cultured with fibroblast growth medium (FGM-2; Clonetics) containing 0.2% FBS, gentamicin-amphotericin B, fibroblast growth factor, and insulin at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. When the cells were grown in subconfluent conditions, the culture medium was replaced with FGM-2 medium without FBS, fibroblast growth factor, and insulin (medium) for 16 hours. The cells were 80% confluent. The density of cell cultures did not change during the culture periods, regardless of culture conditions.

### Western blot analysis of $\alpha$ -smooth muscle actin expression

Expression of  $\alpha$ -smooth muscle actin expression was analyzed by means of Western blotting with an anti- $\alpha$ -smooth muscle actin

antibody (American Research Products, Inc, Belmont, Mass), as described previously.<sup>25,29</sup>

### Measurement of TGF- $\beta_1$

The concentrations of TGF- $\beta_1$  in the culture supernatants from HLFs were measured by using commercially available ELISA kits (Genzyme TECHNE). ELISA was performed according to the manufacturer's instructions. All samples were assayed in duplicate. The minimum detectable concentration of TGF- $\beta_1$  was 7 pg/mL.

### Western blot analysis of JNK, p38 MAP kinase, and Erk

Phosphorylation of JNK, p38 MAP kinase, and Erk was analyzed by means of Western blotting, according to the manufacturer's instructions (New England Biolabs, Inc), by using antibodies to phosphorylated threonine and tyrosine residues of JNK, p38 MAP kinase, and p42/p44 MAP kinase, as described previously.<sup>24</sup> Each antibody reacts specifically with the corresponding kinase.

### JNK, p38 MAP kinase, and Erk activity assay

JNK, p38 MAP kinase, and Erk activities were analyzed with a commercially available SAPK/JNK assay kit, p38 MAP kinase assay kit, and MAP kinase assay kit (New England Biolabs, Inc) according to the manufacturer's instructions, as described previously.<sup>24</sup>

### Statistical analysis

Statistical significance was analyzed by means of ANOVA. A *P* value of less than .05 was considered significant. When statistical significance was reached, post hoc tests (the Fischer protected least-significant difference and Scheffé *F* tests) were performed.

## RESULTS

### IL-4 and IL-13 induce increases in $\alpha$ -smooth muscle actin expression

The expression of  $\alpha$ -smooth muscle actin in IL-4- and IL-13-stimulated HLFs increased in a dose-dependent manner (Fig 1, A-C) and in a time-dependent manner (Fig 1, D-F). We measured the concentrations of TGF- $\beta_1$  in the culture supernatants and examined the effect of anti-TGF- $\beta_1$  antibody on  $\alpha$ -smooth muscle actin expression in unstimulated control HLFs, IL-4-stimulated HLFs, and IL-13-stimulated HLFs to determine the involvement of TGF- $\beta_1$  in  $\alpha$ -smooth muscle actin expression because fibroblasts in culture are known to constitutively produce TGF- $\beta_1$ . TGF- $\beta_1$  concentrations on days 2, 4, and 6 were below the assay sensitivity limit in unstimulated cultures ( $28 \pm 7$  and  $44 \pm 8$  pg/mL, respectively), IL-4-stimulated cultures ( $22 \pm 8$  and  $40 \pm 12$  pg/mL, respectively), and IL-13-stimulated cultures ( $21 \pm 7$  and  $39 \pm 7$  pg/mL, respectively; mean  $\pm$  SD in 3 different experiments). Anti-TGF- $\beta_1$  antibody at sufficient concentrations for neutralizing 10 ng/mL TGF- $\beta_1$  abolished increases in  $\alpha$ -smooth muscle actin expression in unstimulated control HLFs, but not IL-4-stimulated and IL-13-stimulated HLFs, on day 6 (data not shown). These results indicated that TGF- $\beta_1$  constitutively produced by unstimulated control HLFs contributes to increases in  $\alpha$ -smooth muscle actin expression in unstimulated control HLFs but not in IL-4-stimulated and IL-13-stimulated HLFs. Anti-IL-4 antibody at a sufficient concentration for

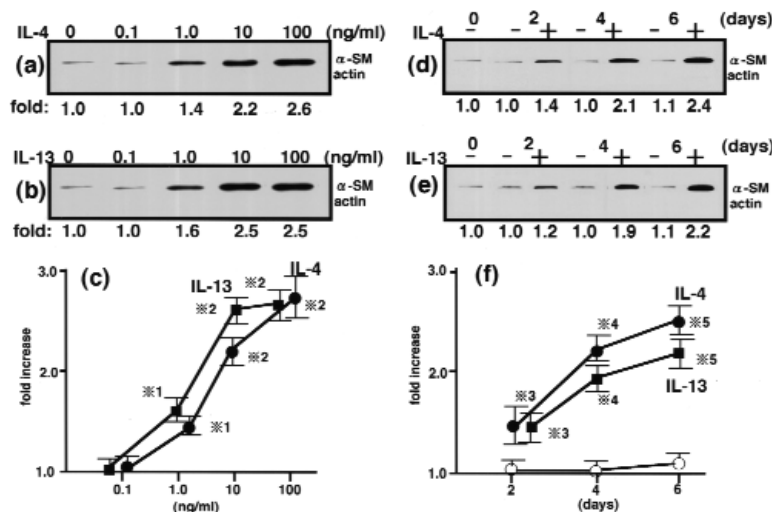


**FIG 1.** IL-4 and IL-13 induce  $\alpha$ -smooth muscle actin expression. HLFs were stimulated with medium or with various concentrations of IL-4 (A) or IL-13 (B) and cultured for 6 days. HLFs were cultured with 100 ng/mL IL-4 (A) or 100 ng/mL IL-13 (B) for 2, 4, and 6 days. The amounts of  $\alpha$ -smooth muscle actin proteins were quantified with the National Institutes of Health image analyzer program and are presented as the amounts of  $\alpha$ -smooth muscle actin proteins relative to those in control cells treated without IL-4 or IL-13 (1.0). The fold increases in amounts of  $\alpha$ -smooth muscle actin proteins are indicated beneath the blot films. Three identical experiments performed independently gave similar results. The results are expressed as means  $\pm$  SD in 3 different experiments of cells cultured with medium (open circles), IL-4 (filled circles), or IL-13 (filled squares; C and F). \*1,  $P < .05$  compared with the amounts of  $\alpha$ -smooth muscle actin proteins in the cells cultured with medium; \*2,  $P < .01$  compared with the amounts of  $\alpha$ -smooth muscle actin proteins in the cells cultured with medium; \*3,  $P < .05$  compared with the amounts of  $\alpha$ -smooth muscle actin proteins in the cells cultured with medium on day 2; \*4,  $P < .01$  compared with the amounts of  $\alpha$ -smooth muscle actin proteins in the cells cultured with medium on day 4; \*5,  $P < .01$  compared with the amounts of  $\alpha$ -smooth muscle actin proteins in the cells cultured with medium on day 6.

neutralizing 500 ng/mL IL-4 abolished increases in  $\alpha$ -smooth muscle actin expression in IL-4-stimulated HLFs on day 6 (data not shown). Similarly, anti-IL-13 antibody at a sufficient concentration for neutralizing 500 ng/mL IL-13 abolished increases in  $\alpha$ -smooth muscle actin expression in IL-13-stimulated HLFs on day 6 (data not shown). These results establish the specificity of the regulatory effect of these cytokines on  $\alpha$ -smooth muscle actin expression. When the cells were stimulated with a combination of TGF- $\beta_1$  (1 ng/mL) and IL-4 (100 ng/mL),  $\alpha$ -smooth muscle actin expression in HLFs was comparable with that found in HLFs stimulated with TGF- $\beta_1$  or IL-4 only. Similar observations were obtained in a combination of TGF- $\beta_1$  (1 ng/mL)-stimulated and IL-4 (100 ng/mL)-stimulated HLFs (data not shown). When the cells were stimulated with a combination of suboptimal concentrations of TGF- $\beta_1$  (0.1 ng/mL) and IL-4 (1 ng/mL), a combination of suboptimal concentrations of TGF- $\beta_1$  (0.1 ng/mL) and IL-13 (1 ng/mL), or a combination of suboptimal concentrations of IL-4 and IL-13 (1 ng/mL), they showed additive effects (data not shown).

#### IL-4 and IL-13 induce JNK and Erk, but not p38 MAP kinase, phosphorylation

Phosphorylation of JNK, p38 MAP kinase, and Erk in the cells was examined at the desired times as indicated.

In IL-4-stimulated cells, amounts of phosphorylated threonine and tyrosine of JNK increased at 15 minutes, were maximal at 30 minutes, and returned to nearly basal levels at 120 minutes (Fig 2, A, upper panel). Amounts of phosphorylated threonine and tyrosine of p38 MAP kinase were not changed at any time during culture (Fig 2, B, upper panel). Amounts of phosphorylated threonine and tyrosine of Erk increased at 15 minutes, were maximal at 30 minutes, and returned to nearly basal levels at 120 minutes (Fig 2, C, upper panel). Similar observations were obtained in IL-13-stimulated cells (Fig 2, D-F). The lower panels of Fig 2, A and D, show that equal amounts of JNK protein were immunoblotted with phosphorylation-independent JNK-specific antibody regardless of time of culture periods, indicating that IL-4- and IL-13-induced JNK phosphorylation occurred in the absence of changes in JNK protein levels. Similarly, IL-4- and IL-13-induced Erk phosphorylation occurred in the absence of changes in Erk protein levels (Fig 2, C and F). The lower panels of Fig 2, B and E, showed that equal amounts of p38 MAP kinase protein were immunoblotted with phosphorylation-independent p38 MAP kinase-specific antibody regardless of time of culture periods, indicating that no changes in IL-4- and IL-13-induced p38 MAP kinase phosphorylation occurred in the absence of changes in p38 MAP kinase protein levels.

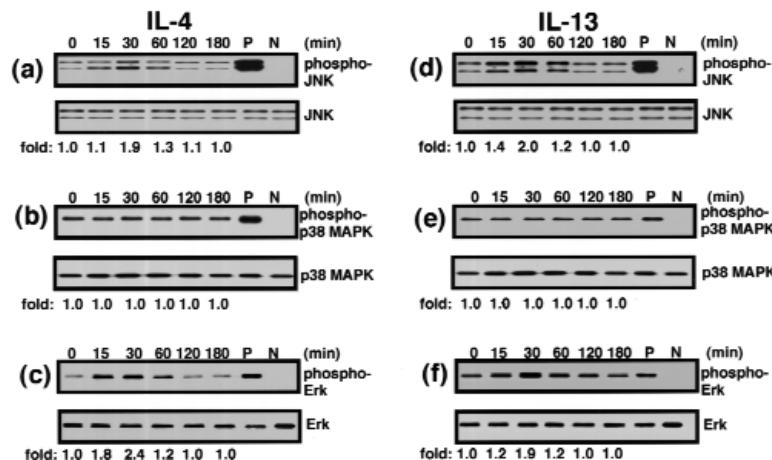


**FIG 2.** IL-4 and IL-13 induce the threonine and tyrosine phosphorylation of JNK and Erk but not p38 MAP kinase. HLFs were stimulated with 100 ng/mL IL-4 (**a**, **b**, and **c**) or 100 ng/mL IL-13 (**d**, **e**, and **f**) for the desired times as indicated. Phosphorylation of JNK, p38 MAP kinase, and Erk was analyzed by means of Western blotting by using antibodies to phosphorylated threonine and tyrosine residues of JNK (*phospho-JNK*; upper panels of **a** and **d**), p38 MAP kinase (*phospho-p38 MAPK*; upper panels of **b** and **e**), or Erk (*phospho-Erk*; upper panels of **c** and **f**). Blots shown in the upper panels of **a** and **d**, **b** and **e**, and **c** and **f** were stripped and reprobed with antibodies to phosphorylation state-independent JNK, p38 MAP kinase, and Erk, respectively, to show the amounts of JNK, p38 MAP kinase, and Erk blotted. Lane **P** of **a**, **b**, and **c** represent phosphorylated JNK, p38 MAP kinase, and Erk control protein for positive control, respectively (New England Biolabs, Inc). Lane **N** of **a**, **b**, and **c** represent nonphosphorylated JNK, p38 MAP kinase, and Erk control protein for negative control, respectively (New England Biolabs, Inc). The amounts of phosphorylated JNK, p38 MAP kinase, and Erk proteins were quantified with the National Institutes of Health image analyzer program and are presented as the amounts of phosphorylated JNK, p38 MAP kinase, and Erk proteins relative to control cells treated without IL-4 and IL-13 (1.0). The fold increases in amounts of phosphorylated JNK, p38 MAP kinase, and Erk proteins are indicated beneath the blot film. Three identical experiments performed independently gave similar results.

### JNK, p38 MAP kinase, and Erk activity and the effect of CEP-1347, SB 203580, and PD98059 on JNK, p38 MAP kinase, and Erk activity

We next examined whether IL-4 and IL-13 could induce increases in JNK, p38 MAP kinase, and Erk activity and the effect of CEP-1347 on JNK activity, SB 203580 on p38 MAP kinase activity, and PD 98059 on Erk activity in IL-4- and IL-13-stimulated HLFs. IL-4 induced increases in JNK activity, as demonstrated by increased phosphorylation of c-Jun (Fig 3, A), and Erk activity, as demonstrated by increased phosphorylation of its substrate, Elk-1 (Fig 3, C). p38 MAP kinase activity, as demonstrated by phosphorylation of its substrate ATF-2, was not increased by IL-4 (Fig 3, B). CEP-1347 and PD 98059 attenuated IL-4-induced increases in JNK (Fig 3, A) and Erk (Fig 3, C) activity, respectively. Similar observations were obtained in IL-13-stimulated cells (Fig 3, D-F). CEP-1347, SB 203580, and PD 98059 did not modulate p38 MAP kinase and Erk activity, JNK and Erk activity, and JNK and p38 MAP kinase activity, respectively, in IL-4-stimulated cells. Similar observations were obtained in IL-13-stimulated cells (data not shown). Addition of DMSO vehicle alone did not attenuate cytokine-induced increases in p38 MAP kinase, Erk, and JNK activity (data not shown).

### CEP-1347, but not SB 203580 and PD 98059, attenuates $\alpha$ -smooth muscle actin

The increases in  $\alpha$ -smooth muscle actin expression and the increases in JNK and Erk phosphorylation and activity were caused by IL-4 and IL-13. These observations suggested that  $\alpha$ -smooth muscle actin expression might be mediated through JNK-dependent and Erk-dependent pathways. To test this possibility, HLFs that had been incubated with various concentrations of CEP-1347 or PD 98059 for 1 hour were stimulated with IL-4 or IL-13, and the expression of  $\alpha$ -smooth muscle actin was examined 6 days after cultivation. In addition, we simultaneously examined the effect of SB 203580 on  $\alpha$ -smooth muscle actin expression in IL-4- and IL-13-stimulated cells to verify the role of p38 MAP kinase. CEP-1347 attenuated IL-4-induced increases in  $\alpha$ -smooth muscle actin expression in a dose-dependent manner (Figs 4, A, and 5, A), whereas SB 203580 and PD 98059 did not attenuate IL-4-induced increases in  $\alpha$ -smooth muscle actin expression (Fig 4, B and C). Similar observations were obtained in IL-13-stimulated cells (Fig 4, D-F, and Fig 5, B). Addition of DMSO vehicle alone did not attenuate  $\alpha$ -smooth muscle actin expression in unstimulated, IL-4-stimulated, and IL-13-stimulated cells (data not shown). The total number of the cells, cell viability determined with trypan blue exclusion

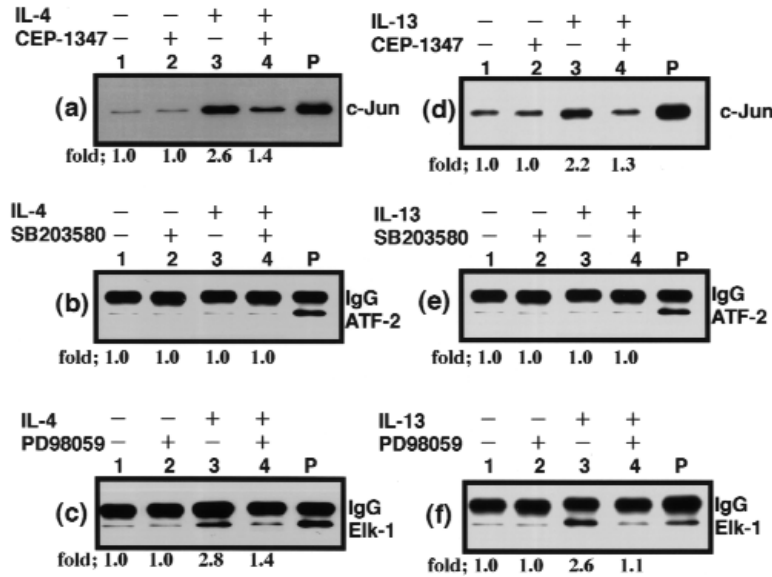


**FIG 3.** IL-4 and IL-13 induce JNK and Erk activity, but not p38 MAP kinase activity, and CEP-1347 and PD 98059 attenuate JNK and Erk activity, respectively. HLFs that had been incubated with medium, CEP-1347 (1  $\mu$ mol/L), SB 203580 (10  $\mu$ mol/L), or PD98059 (50  $\mu$ mol/L) for 1 hour were stimulated with 100 ng/mL IL-4 (A, B, and C) or 100 ng/mL IL-13 (D, E, and F). JNK, p38 MAP kinase, and Erk activity was analyzed at 30 minutes after stimulation. Lane P of A, B, and C represent phosphorylated JNK, p38 MAP kinase, and Erk control protein for positive control, respectively. The amounts of phosphorylated c-Jun, ATF-2, and Elk-1 proteins were quantified with the National Institutes of Health image analyzer program and are presented as the amounts of phosphorylated c-Jun, ATF-2, and Elk-1 proteins relative to control cells treated without IL-4 and IL-13 (1.0). The fold increases in amounts of phosphorylated c-Jun, ATF-2, and Elk-1 proteins are indicated beneath the blot film. Three identical experiments performed independently gave similar results.

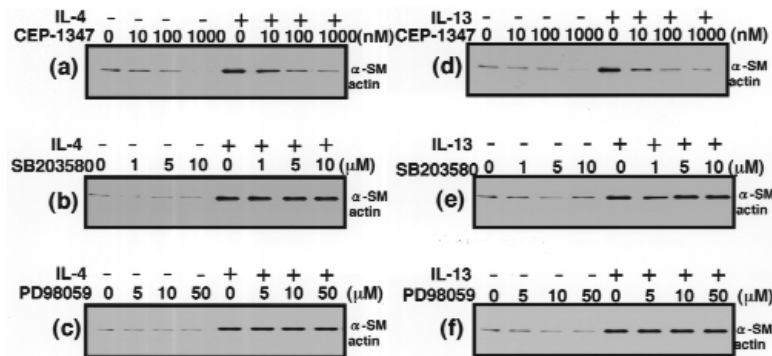


**FIG 4.** CEP-1347, but not SB 203580 and PD 98059, attenuates IL-4- and IL-13-induced  $\alpha$ -smooth muscle actin expression. HLFs that had been incubated with either medium or various concentrations of CEP-1347 (A and D), SB 203580 (B and E), and PD98059 (C and F) for 1 hour were cultured with medium, 100 ng/mL IL-4 (A-C), or 100 ng/mL IL-13 (D-F) for 6 days. Three identical experiments independently performed gave similar results.  $\alpha$ -SM actin,  $\alpha$ -Smooth muscle actin.

dye, and total cell protein at the end of the culture period of each experiment indicated in Figs 1 through 5 did not differ with culture conditions (data not shown).

## DISCUSSION

In the present study we examined the role of IL-4 and IL-13 in inducing the phenotypic modulation of HLFs to myofibroblasts and analyzed the intracellular signal-induc-

ing myofibroblastic phenotype of HLF characterized by the expression of  $\alpha$ -smooth muscle actin. The results showed the following: (1) IL-4 and IL-13 induced the phenotypic modulation of HLFs to myofibroblasts in a dose- and time-dependent manner; (2) anti-TGF- $\beta_1$  antibody did not affect IL-4- and IL-13-induced phenotypic modulation of HLFs to myofibroblasts, and the concentrations of TGF- $\beta_1$  in the IL-4- and IL-13-stimulated cultures were below the assay sensitivity limits; (3) IL-4 and IL-13 induced increases in

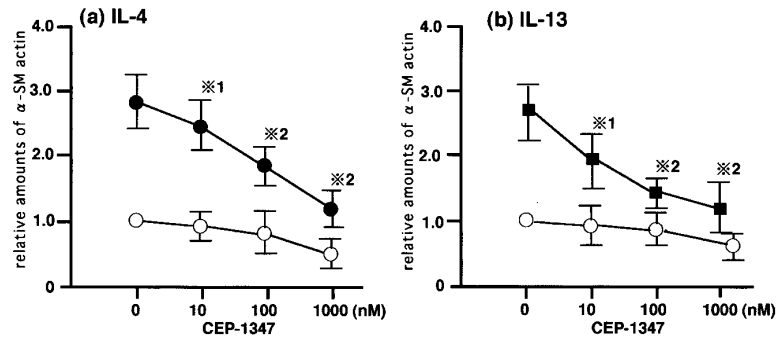


**FIG 5.** CEP-1347 attenuates IL-4- and IL-13-induced  $\alpha$ -smooth muscle actin expression. The amounts of  $\alpha$ -smooth muscle actin proteins shown in Fig 4, A and D, were quantified with the National Institutes of Health image analyzer program, and the relative amounts of  $\alpha$ -smooth muscle actin proteins were calculated as the amounts of  $\alpha$ -smooth muscle actin proteins relative to control cells treated without IL-4 and IL-13 (1.0). The results are expressed as means  $\pm$  SD of the relative amounts of  $\alpha$ -smooth muscle actin proteins in cells cultured with medium (open circles), IL-4 (filled circles), or IL-13 (filled squares) in 3 different experiments. \*1,  $P < .05$  compared with the amounts of  $\alpha$ -smooth muscle actin proteins in IL-4- or IL-13-stimulated cells cultured without CEP-1347; \*2,  $P < .01$  compared with the amounts of  $\alpha$ -smooth muscle actin proteins in IL-4- or IL-13-stimulated cells cultured without CEP-1347.

JNK and Erk phosphorylation and activity but not p38 MAP kinase activity; (4) CEP-1347 and PD 98059 attenuated IL-4- and IL-13-induced JNK and Erk activity, respectively; and (5) CEP-1347 attenuated IL-4- and IL-13-induced phenotypic modulation of HLFs to myofibroblasts in a dose-dependent manner. These results indicate that IL-4 and IL-13 are capable of inducing the myofibroblastic phenotype of HLFs, and JNK regulates the phenotypic modulation of HLFs to myofibroblasts in IL-4- and IL-13-stimulated HLFs.

It has been demonstrated that the number of myofibroblasts present in the submucosa correlates with subepithelial collagen deposition in bronchial asthma.<sup>4,7</sup> Thus myofibroblasts play a role in the airway remodeling response of asthma. Several cell types present in the lung are potential candidates as the origin of myofibroblasts, and myofibroblasts appear to originate from fibroblasts under the influence of various factors.<sup>30,31</sup> Among these factors, TGF- $\beta_1$  is well known to induce the phenotypic modulation of fibroblasts to myofibroblasts.<sup>29</sup> An increased concentration of TGF- $\beta_1$  in bronchoalveolar lavage fluid and an increased expression of TGF- $\beta_1$  in the airway epithelium and submucosa of asthmatic subjects have been shown,<sup>32,33</sup> and thus TGF- $\beta_1$  may be involved in the phenotypic modulation of lung fibroblasts to myofibroblasts in the airway remodeling response of asthma. It is generally accepted that the airway remodeling response is preceded by allergic inflammation, and T<sub>H</sub>2 cytokines, such as IL-4 and IL-13, play a key role in the production of allergic inflammation.<sup>10,34</sup> However, the role of IL-4 and IL-13 in the development of airway remodeling has not been clarified. In addition, although IL-4 and IL-13 can activate fibroblasts to elicit a variety of cellular functions,<sup>13-17</sup> the role of these cytokines in inducing phenotypic modulation of fibroblasts to myofibroblasts has not been determined. In the present study we showed that IL-4 and IL-13 induced the phenotypic

modulation of HLFs to myofibroblasts. IL-4 is reported to induce the myofibroblastic phenotype of synovial fibroblasts from patients with osteoarthritis<sup>35</sup>; however, the role of IL-4 and IL-13 in inducing myofibroblastic phenotype of HLFs has never been determined. The present results with IL-4- and IL-13-mediated induction of phenotypic modulation of fibroblasts to myofibroblasts indicate the role of IL-4 and IL-13 in airway remodeling response of asthma.

In the present study we analyzed the role of the MAP kinase superfamily in IL-4- and IL-13-induced  $\alpha$ -smooth muscle actin expression in HLF. IL-4 and IL-13 activated JNK and Erk but not p38 MAP kinase. The specific inhibitors of the p38 MAP kinase and Erk signaling pathways have been identified, providing effective tools for investigating the role of p38 MAP kinase and Erk in cellular signaling.<sup>27,28</sup> CEP-1347 is a novel inhibitor of the JNK signaling pathway.<sup>26</sup> In the present study we used these inhibitors to elucidate the biologic functions of p38 MAP kinase, Erk, and/or JNK in  $\alpha$ -smooth muscle actin expression in IL-4- and IL-13-stimulated HLFs. Concentrations up to 10  $\mu$ mol/L SB 203580 and up to 50  $\mu$ mol/L PD 98059 were used in this study to examine the inhibitory effect of these inhibitors on  $\alpha$ -smooth muscle actin expression because the previous studies of the role of p38 MAP kinase and Erk in eliciting various biologic responses had shown that 10  $\mu$ mol/L SB 203580 and 50  $\mu$ mol/L PD 98059 caused almost complete inhibition.<sup>27,28,36,37</sup> Concentrations of 1  $\mu$ mol/L CEP-1347 can rescue motoneurons undergoing apoptosis and inhibit JNK activity.<sup>26</sup> Consequently, concentrations of 10  $\mu$ mol/L SB 203580, 50  $\mu$ mol/L PD 98059, and 1  $\mu$ mol/L CEP-1347, as used in this study, were sufficient to examine the signal transduction pathway. IL-4 and IL-13 simultaneously induced JNK and Erk activity but not p38 MAP kinase activity. Concentrations of 1  $\mu$ mol/L CEP-1347 and 50  $\mu$ mol/L PD 98059 almost completely attenuated IL-

4-induced increases in JNK and Erk activity, respectively. A concentration of 1  $\mu\text{mol/L}$  CEP-1347, but not 10  $\mu\text{mol/L}$  SB 203580 or 50  $\mu\text{mol/L}$  PD 98059, almost completely attenuated IL-4-induced  $\alpha$ -smooth muscle actin expression. Similar observations were obtained in IL-13-stimulated cells. These results indicated that JNK, but not p38 MAP kinase and Erk, participates in regulating IL-4- and IL-13-induced phenotypic modulation of HLFs to myofibroblasts, as characterized by  $\alpha$ -smooth muscle actin expression.

IL-4 shares many functional activities with IL-13 and uses similar receptor subunits for signaling.<sup>11,34</sup> IL-4 and IL-13 modulate various cell functions used by various intracellular signals. IL-4 activates the Janus kinase and signal transducer and activator of transcription 6 pathways to induce the development of  $T_H2$  cells,<sup>38</sup> the anti-inflammatory effect of IL-4,<sup>39</sup> and P-selectin expression.<sup>16</sup> IL-4-activated phosphatidylinositol-3' kinase mediates antiapoptotic effects.<sup>40</sup> With regard to the MAP kinase superfamily, the effect of IL-4 on MAP kinase activation is cell-type specific. IL-4 activates Erk in human keratinocytes and hepatoma cells<sup>41,42</sup> and JNK in a hematopoietic cell line, Ba/F3.<sup>43</sup> By contrast, IL-4 fails to activate Erk and p38 MAP kinase in mast cells and T lymphocytes<sup>44,45</sup> and JNK in a hematopoietic cell line, MC/9.<sup>46</sup> In addition, the inhibition by IL-4 of Erk activation in monocytes and macrophages is reported.<sup>47,48</sup> In the present study we showed that IL-4- and IL-13-activated JNK mediates the phenotypic modulation of HLFs to myofibroblasts. These results indicate a novel biologic function of JNK in IL-4- and IL-13-activated intracellular signal transduction pathway in fibroblasts.

From the data presented here, we conclude that the  $T_H2$  cytokines IL-4 and IL-13 play a role in the production of airway remodeling by means of inducing the myofibroblastic phenotype of HLFs, as well as allergic inflammation, and that JNK regulates the phenotypic modulation of HLFs to myofibroblasts. Our results concerning the role of the JNK-dependent pathway in IL-4- and IL-13-induced phenotypic modulation of HLFs to myofibroblasts are important in understanding the airway remodeling process of bronchial asthma, and a strategy of attenuating phenotypic modulation of HLFs to myofibroblasts by using the specific inhibitor of JNK cascade may produce beneficial effects in preventing development of airway remodeling.

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## Correction

In the article in the April issue by Lacy et al, "Fusion protein vesicle-associated membrane protein 2 is implicated in IFN- $\gamma$ -induced piecemeal degranulation in human eosinophils from atopic individuals" (2001;107:671-8), the following funding information should have been included: "This work was supported by the Medical Research Council, Canada, and the Alberta Heritage Foundation for Medical Research. Paige Lacy, PhD, is a Parker B. Francis Fellow in Pulmonary Research; Redwan Moqbel, PhD, FRCPath, is an Alberta Heritage Senior Medical Scholar."