

Original Article

Interleukin-4 and interleukin-13 induce fibronectin production by human lung fibroblasts

Tatsuya Machino, Shu Hashimoto, Yasuhiro Gon, Kosei Kujime, Shuichiro Maruoka and Takashi Horie

First Department of Internal Medicine Nihon University School of Medicine, Tokyo, Japan

ABSTRACT

Background: Interleukin (IL)-4 and IL-13, which play a role in the production of allergic inflammation, can activate fibroblasts to elicit a variety of cellular functions. However, the effect of IL-4 and IL-13 on fibronectin production by human lung fibroblasts (HLF) has not been determined. In the present study, we examined the effect of IL-4 and IL-13 on fibronectin production by HLF in order to clarify these issues.

Methods: We stimulated HLF with various concentrations of IL-4 and IL-13 for 12, 24, 48 and 72 h and fibronectin concentrations in the culture supernatants were determined by ELISA.

Results: The results showed that IL-4 and IL-13 induced fibronectin production in a time- and a concentration-dependent manner. Anti-transforming growth factor (TGF)- β_1 antibody (ab) inhibited TGF- β_1 -induced fibronectin production but not IL-4- and IL-13-induced fibronectin production, indicating that TGF- β_1 is not involved in the IL-4- and IL-13-induced fibronectin production by HLF.

Conclusions: These results indicate that IL-4 and IL-13 are capable of inducing fibronectin production by HLF and play an important role in the production of airway remodeling by inducing fibronectin production as well as allergic inflammation.

Key words: fibronectin, human lung fibroblast, interleukin-4, interleukin-13, remodeling.

INTRODUCTION

Airway remodeling has been documented to be a prominent feature of the bronchial wall of patients with chronic severe asthma.^{1–3} Many elements contribute to the airway remodeling response, which involves basement membrane thickening associated with subepithelial extracellular matrix deposition and subepithelial fibrosis.^{1–3} Bronchial asthma is a disease that is characterized by episodic reversible airway obstruction, airway hyper-responsiveness and allergic inflammation in the airway.⁴ T helper (h) 2 cytokines play a key role in the production of allergic inflammation of asthma.⁵ Of the Th2 cytokines, it has been demonstrated that the interleukin (IL)-4 and IL-13 receptors are expressed in fibroblasts^{6,7} and that IL-4 and IL-13 elicit various fibroblast cell functions, including cytokine and adhesion molecule expression and extracellular matrix protein synthesis.^{8–12} These observations indicate that IL-4 and IL-13 play a role in the airway remodeling response of asthma. Interleukin-4 has been shown to induce fibronectin production by human synovial fibroblasts from patients with rheumatoid arthritis and osteoarthritis and infant dermal fibroblasts.⁸ However, little is known about the effect of IL-4 on fibronectin production by lung fibroblasts derived from healthy subjects. Furthermore, the role of IL-13 in fibronectin production has not been determined. Therefore, in the present study clarified the role of IL-4 and IL-13 in fibronectin production by examining the role of IL-4 and IL-13 in fibronectin production by normal human lung fibroblasts (HLF). To this end, we attempted to examine the effect of IL-13 as well as IL-4 on fibronectin production by HLF.

Correspondence: Dr Shu Hashimoto, First Department of Internal Medicine, Nihon University School of Medicine, 30-1 Oyaguchikamimachi, Itabashi-ku, Tokyo 173-8610, Japan. Email: shuh@med.nihon-u.ac.jp

Received 16 October 2000. Accepted for publication 1 March 2001.

METHODS

Cells and reagents

Human lung fibroblasts were obtained from Clonetics (San Diego, CA, USA). Human lung fibroblasts were grown in a culture medium that was fibroblast growth medium (FGM-2; Clonetics) containing 0.2% fetal bovine serum (FBS), gentamycin–amphotericin B, fibroblast growth factor (FGF) and insulin. Interleukin-4 and IL-13 were obtained from Endogen (Woburn, CA, USA) and R&D Systems (Minneapolis, MN, USA), respectively. Anti-transforming growth factor (TGF)- β_1 antibody (ab; clone 9016.2, mouse IgG1), anti-IL-13 ab (clone 32116.11, mouse IgG1) and IL-5 were obtained from Genzyme Techne (Minneapolis, MN, USA). The anti-IL-4 ab (clone 34019.111, mouse IgG2b) and isotype-matched control mouse IgG1 and IgG2 were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Cell cultures

For the determination of fibronectin production, cells were placed onto collagen-coated 24-well flat-bottomed tissue culture plates (Iwaki, Tokyo, Japan) and cultured using culture medium at 37°C in a humidified 5% CO₂ atmosphere. When cells had grown to subconfluency, the culture medium was replaced with FGM-2 medium without FBS, FGF and insulin (growth factor-free medium) for 16 h. In order to determine fibronectin production by IL-4- and IL-13-stimulated HLF, cells were stimulated with various concentrations of IL-4 and IL-13 and cultured for various lengths of time, as indicated. To examine the effects of anti-TGF- β_1 ab on IL-4- and IL-13-induced fibronectin production, cells that had been incubated with anti-TGF- β_1 ab for 1 h were stimulated with IL-4 or IL-13 and cultured.

Measurement of fibronectin and TGF- β_1

The concentrations of fibronectin in the culture supernatants from HLF were measured by commercially available ELISA kits (Chemicon International, Temecula, CA, USA). The concentrations of TGF- β_1 in culture supernatants from HLF were measured by commercially available ELISA kits (Genzyme Techne). The minimum detectable concentration of TGF- β_1 was 7 pg/mL. The ELISA was performed according to the manufacturer's instructions. All samples were assayed in duplicate.

Statistical analysis

Statistical significance was analyzed using analysis of variance (ANOVA). $P < 0.05$ was considered significant.

RESULTS

Interleukin-4 and IL-13 induce fibronectin production in a time-dependent manner

First, we examined the time-dependent effects of IL-4 and IL-13 on fibronectin production by HLF. To this end, the culture supernatants from HLF stimulated with 100 ng/mL IL-4 or 100 ng/mL IL-13 were harvested at 12, 24, 48 and 72 h after cultivation. The concentration of fibronectin in the culture supernatants from IL-4-stimulated HLF increased at 12 h, was maximal at 48 h and decreased at 72 h (Fig. 1). Similarly, the concentration of fibronectin in culture supernatants from IL-13-stimulated HLF increased in a time-dependent manner (Fig. 1). In order to establish the specificity of the regulatory effect of IL-4 and IL-13 on fibronectin production, HLF that had been incubated with anti-IL-4 ab or anti-IL-13 ab were stimulated with IL-4 or IL-13 and the concentration of fibronectin in the culture supernatants

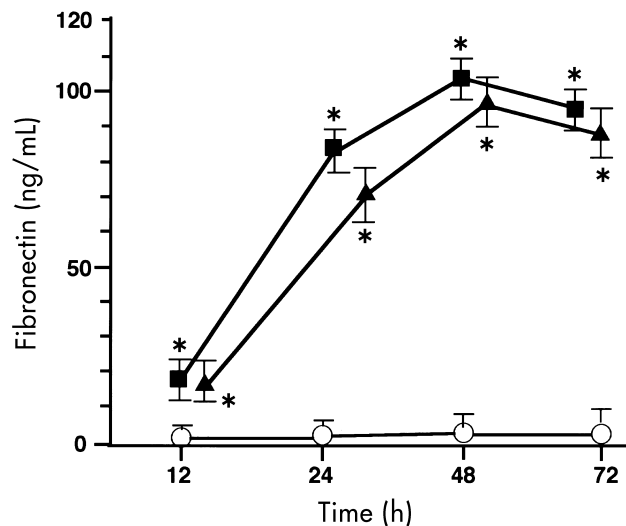


Fig. 1 Interleukin (IL)-4 and IL-13 induce fibronectin production in a time-dependent manner. Human lung fibroblasts (HLF) were cultured with medium (○), 100 ng/mL IL-4 (■) or 100 ng/mL IL-13 (▲) and cultured for 12, 24 and 48 h. The concentration of fibronectin in culture supernatants was determined by ELISA. The results are expressed as the mean \pm SD of five experiments. * $P < 0.01$ compared with the concentration of fibronectin in culture supernatants from HLF cultured with medium.

was then determined at 48 h. At 50 $\mu\text{g}/\text{mL}$ anti-IL-4 ab, a concentration that was sufficient for neutralizing IL-4 activity (500 ng/mL), fibronectin production by IL-4-stimulated HLF was abolished. Similarly, 50 $\mu\text{g}/\text{mL}$ anti-IL-13 ab, which was sufficient for neutralizing IL-13 activity (500 ng/mL), abolished fibronectin production by IL-13-stimulated HLF. Isotype-matched control mouse IgG had no effect on fibronectin production by IL-4- and IL-13-stimulated HLF (data not shown). These results establish the specificity of the regulatory effect of these cytokines on fibronectin production.

IL-4 and IL-13 induce fibronectin production in a concentration-dependent manner

In the next series of experiments, we examined the concentration-dependent effects of IL-4 and IL-13 on fibronectin production by HLF. To this end, culture supernatants from HLF stimulated with various concentrations of IL-4 or IL-13 were harvested at 48 h after cultivation. The concentration of fibronectin in culture supernatants from IL-4-stimulated HLF increased in a concentration-dependent manner when HLF were stimulated with 0.1–100 ng/mL IL-4 (Fig. 2). When HLF were stimulated with 500 ng/mL IL-4, fibronectin production was not

enhanced further. Similar observations were obtained for IL-13-stimulated HLF (Fig. 2). Interleukin-5 did not induce fibronectin production by HLF (data not shown).

Transforming growth factor- β_1 induces fibronectin production and a combination of TGF- β_1 and IL-4 or IL-13 additively induces fibronectin production

The concentration of fibronectin in culture supernatants from TGF- β_1 -stimulated HLF at 48 h after cultivation increased in a concentration-dependent manner (Fig. 3). When cells were stimulated with a combination of TGF- β_1 (10 ng/mL) and IL-4 (100 ng/mL), TGF- β_1 and IL-4 additively induced fibronectin production (Fig. 4). Similar observations were obtained for HLF cultures stimulated with TGF- β_1 (10 ng/mL) and IL-13 (100 ng/mL; Fig. 4). In contrast, when cells were stimulated with a combination of IL-4 (100 ng/mL) and IL-13 (100 ng/mL), the concentration of fibronectin in culture supernatants from HLF stimulated with IL-4 and IL-13 was comparable to that from HLF stimulated with IL-4 or IL-13 alone (Fig. 4). Human lung fibroblasts were stimulated with TGF- β_1 (10 ng/mL) in the presence of anti-TGF- β_1 ab and it was observed that anti-TGF- β_1 ab abolished the effects of

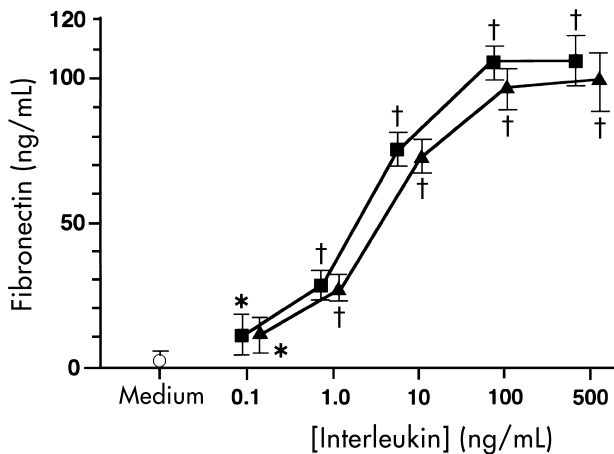


Fig. 2 Interleukin (IL)-4 and IL-13 induce fibronectin production in a concentration-dependent manner. Human lung fibroblasts (HLF) were cultured for 48 h with medium (○) or various concentrations of IL-4 (■) or IL-13 (▲). The concentration of fibronectin in culture supernatants was determined by ELISA. The results are expressed as the mean \pm SD of five experiments. * $P < 0.05$, † $P < 0.01$ compared with fibronectin concentrations in culture supernatants from HLF cultured with medium.

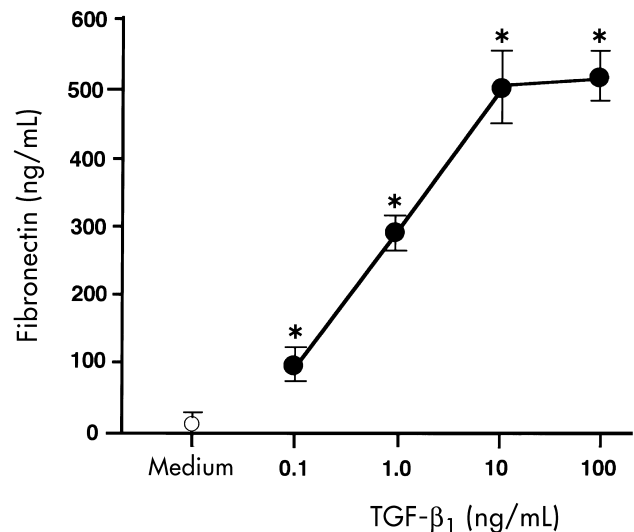


Fig. 3 Transforming growth factor (TGF)- β_1 induces fibronectin production. Human lung fibroblasts (HLF) were cultured for 48 h with medium (○) or various concentrations of TGF- β_1 (●). The concentration of fibronectin in culture supernatants was determined by ELISA. The results are expressed as the mean \pm SD of five experiments. * $P < 0.01$ compared with fibronectin concentrations in culture supernatants from HLF cultured with medium.

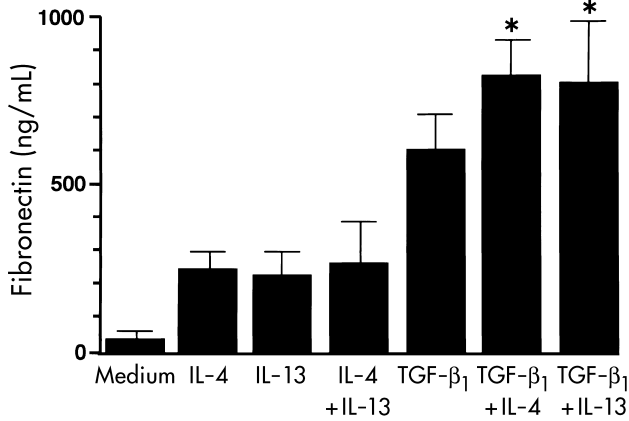


Fig. 4 A combination of transforming growth factor (TGF)-β₁ and interleukin (IL)-4 or IL-13 additively induces fibronectin production. Human lung fibroblasts (HLF) were stimulated with TGF-β₁ (10 ng/mL) and either IL-4 (100 ng/mL) or IL-13 (100 ng/mL) and cultured for 48 h. The concentration of fibronectin in culture supernatants was determined by ELISA. The results are expressed as the mean ± SD of five experiments. **P* < 0.01 compared with fibronectin concentrations in culture supernatants from HLF cultured with IL-4 or IL-13 alone and *P* < 0.05 compared with fibronectin concentrations in culture supernatants from HLF cultured with TGF-β₁ only.

TGF-β₁ on fibronectin production. When HLF were stimulated with IL-4 (100 ng/mL) or IL-13 (100 ng/mL) in the presence of anti-TGF-β₁ ab, anti-TGF-β₁ ab did not affect IL-4- and IL-13-induced fibronectin production (Fig. 5). In addition, the concentration of TGF-β₁ in IL-4- and IL-13-stimulated cultures was below the sensitivity limits of the assay (data not shown). The concentration of anti-TGF-β₁ ab used in the present study (100 μg/mL) was sufficient to neutralize TGF-β₁ activity (100 ng/mL). Isotype-matched control mouse IgG had no effect on fibronectin production by TGF-β₁-, IL-4- and IL-13-stimulated HLF (data not shown).

DISCUSSION

In the present study, we attempted to clarify the role of IL-4 and IL-13 in airway remodeling by examining the effect of IL-4 and IL-13 on fibronectin production by HLF. We focused on the effect of these cytokines on fibronectin production by HLF because an increased deposition of fibronectin has been shown at the site of airway remodeling.^{1,13} The results of the present study showed that IL-4 induced fibronectin production in a time- and

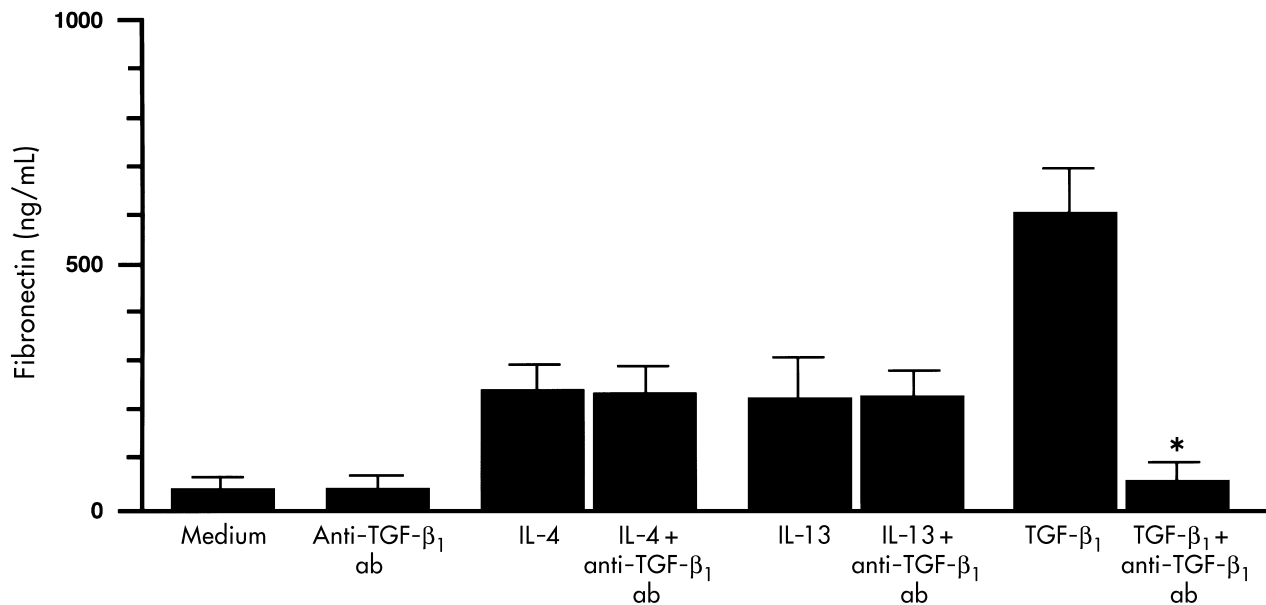


Fig. 5 Anti-transforming growth factor (TGF)-β₁ antibody (ab) inhibits TGF-β₁- but not IL-4- and IL-13-induced fibronectin production. Human lung fibroblasts (HLF) that had been incubated with a sufficient concentrations of anti-TGF-β₁ ab for neutralizing TGF-β₁ were stimulated with TGF-β₁ (10 ng/mL), IL-4 (100 ng/mL) or IL-13 (100 ng/mL) and cultured for 48 h. The concentration of fibronectin in culture supernatants was determined by ELISA. The results are expressed as the mean ± SD of five experiments. **P* < 0.01 compared with fibronectin concentrations in culture supernatants from HLF cultured with TGF-β₁.

concentration-dependent manner. Similar observations were obtained for IL-13-stimulated HLF. Interleukin-13 shares many functional activities with IL-4 and uses similar receptor subunits for signaling.^{6,14} Interleukin-4 has been shown to induce fibronectin production by fibroblastic cell lines.⁸ The results of the present study showed that IL-4 was capable of inducing fibronectin production by HLF as well as fibroblastic cell lines and that IL-13 had a similar activity to IL-4 in inducing fibronectin production by HLF. In addition, when HLF were stimulated with a combination of IL-4 and IL-13, an additive effect was not seen. These results indicate that IL-4 and IL-13 use a common pathway to induce fibronectin production by HLF.

Because TGF- β_1 is known to induce fibronectin production by fibroblasts,^{15,16} TGF- β_1 may be responsible for the induction of fibronectin production by IL-4- and IL-13-stimulated HLF. To test this possibility, we examined the effect of anti-TGF- β_1 ab on fibronectin production by IL-4- and IL-13-stimulated cultures and measured TGF- β_1 concentrations in IL-4- and IL-13-stimulated cultures. The results were as follows: (i) anti-TGF- β_1 ab did not affect IL-4- and IL-13-induced fibronectin production; and (ii) the concentration of TGF- β_1 in IL-4- and IL-13-stimulated cultures was below the sensitivity limits of the assay. These results indicate that IL-4 and IL-13 are capable of inducing fibronectin production by HLF and that TGF- β_1 is not involved in the IL-4- and IL-13-induced production of fibronectin by HLF.

It is generally accepted that the airway remodeling response is preceded by allergic inflammation and that Th2 cytokines, such as IL-4 and IL-13, play a key role in the production of allergic inflammation.^{5,13} Several lines of study have suggested that IL-4 and IL-13 play a role in the development of airway remodeling. Interleukin-4 and IL-13 can activate fibroblasts to elicit a variety of cellular functions, including extracellular matrix production.⁸⁻¹² Pulmonary overexpression of IL-13 causes allergic inflammation and subepithelial fibrosis¹⁷ and IL-13 inhibitors block the development of fibrogenic process.¹⁸ In addition to this evidence, the present results with IL-4- and IL-13-induced production of fibronectin by HLF indicate that IL-4 and IL-13 may play a role in the development of fibrogenic processes in airway remodeling by inducing fibronectin production by HLF. We simultaneously examined the effect of another Th2 cytokine, namely IL-5, on fibronectin production by HLF. Interleukin-5 did not induce fibronectin production, indicating that IL-4 and IL-13, but not all Th2 cytokines, can induce fibronectin production by HLF.

The pathogenesis of airway remodeling is complex. Multiple inflammatory cells, cytokines and mediators participate in the pathogenesis of airway remodeling. Fibroblasts are involved in the development of fibrogenic process in airway remodeling by producing extracellular matrix. Therefore, an understanding of the mechanism of fibronectin production would suggest a strategy for therapy to control the development of fibrogenic processes in airway remodeling in bronchial asthma.

From the data presented here, we conclude that the Th2 cytokines IL-4 and IL-13 play an important role in the production of airway remodeling by inducing fibronectin production by HLF, as well as allergic inflammation.

ACKNOWLEDGMENT

This work was financially supported, in part, by a Grant-in-Aid for High-Tech Research Center from the Japanese Ministry of Education, Science, Sports, and Culture to Nihon University.

REFERENCES

- 1 Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. *J. Clin. Invest.* 1999; **104**: 1001–6.
- 2 Busse W, Elisa J, Sheppard D, Banks-Schlegel S. Airway remodeling and repair. *Am. J. Respir. Crit. Care Med.* 1999; **160**: 1035–42.
- 3 Fish JE, Peters SP. Airway remodeling and persistent airway obstruction in asthma. *J. Allergy Clin. Immunol.* 1999; **104**: 509–16.
- 4 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am. Rev. Respir. Dis.* 1987; **142**: 225–44.
- 5 Chung KF, Barnes PJ. Cytokines in asthma. *Thorax* 1999; **54**: 825–57.
- 6 Murata T, Husain SR, Mohri H, Puri RK. Two different IL-13 receptor chains are expressed in normal human skin fibroblasts, and IL-4 and IL-13 mediate signal transduction through a common pathway. *Int. Immunol.* 1998; **10**: 1103–10.
- 7 Feng N, Lugli SM, Schneder B *et al.* The intereukin-4/interleukin-13 receptor of human synovial fibroblasts: Overexpression of the nonsignaling interleukin-13 receptor $\alpha 2$. *Lab. Invest.* 1998; **78**: 591–602.
- 8 Postlethwaite AE, Holness MA, Katani H, Raghov R. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin-4. *J. Clin. Invest.* 1992; **90**: 1479–85.
- 9 Doucet CD, Brouty-Boye D, Pottin-Celmeceau C, Jasmin C, Canonica GW, Azzarone B. IL-4 and IL-13 specifically increase adhesion molecule and inflammatory cytokine

- expression in human lung fibroblasts. *Int. Immunol.* 1998; **10**: 1421–33.
- 10 Doucet C, Brouty-Boye D, Pottin-Clemenceau C, Canonica GW, Jasmin C, Azzarone B. Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. *J. Clin. Invest.* 1998; **101**: 2129–39.
 - 11 Khew-Goodall Y, Wadham C, Stein BN, Gamble JR, Vadas MA. Stat6 activation is essential for interleukin-4 induction of P-selectin transcription in human umbilical vein endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 1999; **19**: 1421–9.
 - 12 Spoelstra FM, Postma DS, Hovenga H, Noordhoek JA, Kaufman HF. Interferon- γ and interleukin-4 differentially regulate ICAM-1 and VCAM-1 expression on human lung fibroblasts. *Eur. Respir. J.* 1999; **14**: 759–66.
 - 13 Roche WR, Beasley R, Williams JH, Holgate ST. Sub-epithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; **i**: 520–4.
 - 14 de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory response. *J. Allergy Clin. Immunol.* 1998; **102**: 165–9.
 - 15 Kovacs EJ, DiPietro LA. Fibrogenic cytokines and connective tissue production. *FASEB J.* 1994; **8**: 854–61.
 - 16 Hocevar BA, Brown TL, Howe PH. TGF- β induces fibronectin synthesis through a c-Jun-N-terminal kinase-dependent, Smad4-independent pathway. *EMBO J.* 1999; **18**: 1345–56.
 - 17 Zhu Z, Homer RJ, Wang Z *et al.* Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest.* 1999; **103**: 779–88.
 - 18 Chiamontre MG, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J. Clin. Invest.* 1999; **104**: 777–85.