

Mast cells regulate procollagen I (α_1) production by bronchial fibroblasts derived from subjects with asthma through IL-4/IL-4 δ 2 ratio

Sophie Plante, BSc,^a AbdelHabib Semlali, PhD,^a Philippe Joubert, PhD,^b
Élyse Bissonnette, PhD,^a Michel Laviolette, MD,^a Qutayba Hamid, MD, PhD,^b
and Jamila Chakir, PhD^a *Sainte-Foy and Montreal, Quebec, Canada*

Background: Asthma is characterized by inflammation and remodeling. Mast cells are generally increased in bronchial mucosa of subjects with asthma. These cells release a wide variety of cytokines and mediators that have the capacity to stimulate other resident cells such as smooth muscle cells and fibroblasts.

Objective: This study was designed to evaluate whether mast cells modulate collagen production by bronchial fibroblasts isolated from subjects with asthma and normal subjects through cytokine production.

Methods: Human mast cells were cocultured for 72 hours with primary bronchial fibroblasts isolated from bronchial biopsies of subjects with mild asthma and normal controls. Procollagen I (α_1), IL-4R α , IL-13R α_1 , and IL-13R α_2 gene expression by bronchial fibroblasts and IL-4 and IL-4 δ 2 gene expression by mast cells were quantified by real-time RT-PCR. IL-4 production was also measured by ELISA in culture supernatants.

Results: Procollagen I (α_1) gene expression by fibroblasts from subjects with asthma was significantly higher compared with cells from normal controls when cocultured with mast cells. Mast cells expressed IL-4 isoform and IL-4 δ 2, an alternative splice variant of IL-4. Coculture significantly increased the expression of IL-4 but not IL-4 δ 2 by mast cells when they were cultured with fibroblasts from subjects with asthma compared with cells from normal controls. Neutralization of IL-4 abrogated collagen mRNA expression. There was no significant change in IL-4R α or IL-13R α_1 . However, IL-13R α_2 gene expression was significantly reduced in fibroblasts from subjects with asthma.

Conclusion: These results suggest that inflammatory process may regulate airway remodelling through crosstalk between

inflammatory and structural cells. Targeting this crosstalk may have therapeutic application.

Clinical implications: Understanding mechanisms that govern airway remodeling and collagen deposition in asthma is a step toward therapeutic management of this disease. In this work, we found that mast cell-fibroblast crosstalk may be a potential future target to control some aspects of airway remodeling. (J Allergy Clin Immunol 2006;117:1321-7.)

Key words: Asthma, collagen, fibroblasts, mast cells, remodeling

Mast cells are effector cells in allergic diseases such as asthma and rhinitis. Furthermore, they play an important role in host defence against bacterial infections and may participate to fibrotic processes.¹⁻³ Through their capacity to produce different mediators, a wide variety of tissue responses result. Mast cells are potent source of preformed lipid mediators as well as a large number of cytokines and chemokines.⁴

Evidence suggests that mast cells participate not only in inflammatory responses but also in tissue remodeling and fibrotic processes occurring in many tissues.⁵ Mast cells are usually present at sites of fibrosis such as the skin in scleroderma and the pulmonary tissue in fibrotic lung disorders.^{6,7} Experimental evidence suggests that mast cells modulate fibroblast functions. They induce skin fibroblasts to proliferate and synthesize increased amounts of collagen.⁸ Furthermore, rat mast cells increased fibroblast metalloproteinase 2 and pro-metalloproteinase 2 activity.⁹ Human mast cells are also a source of basic fibroblast growth factor, a potent mitogenic factor for fibroblasts.¹⁰

Airway remodeling in asthma is characterized by subepithelial fibrosis caused by extensive deposition of extracellular matrix. Ultrastructural and immunohistologic studies of bronchial biopsies from subjects with asthma revealed apparent basement membrane thickening caused by the deposition of fibrillar collagen beneath the epithelial basal lamina.¹¹ Fibronectin and type I, III, and V collagens are the major constituents of this subepithelial fibrosis.¹² It has also been shown that bronchial subepithelial fibrosis observed in asthma correlates with the number of myofibroblasts.¹³

In asthma, mast cell number is increased in bronchial biopsies.¹⁴ These cells are predominantly activated by IgE receptor crosslink. In response to activation, preformed mediators that are stored such as TNF, IL-4, IL-13, histamine, and tryptase are released.¹⁵ Tryptase I has been

From ^athe Centre de recherche de l'Hôpital Laval, Institut universitaire de cardiologie et de pneumologie, Sainte-Foy; and ^bMeakins-Christie Laboratories, McGill University, Montreal.

Supported by grants from Canadian Institutes of Health Research and Le Réseau du Fonds de la Recherche en Santé du Québec en Santé Respiratoire. Dr Chakir is recipient of Chercheur-Boursier awards from the Fonds de la Recherche en Santé du Québec.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication March 14, 2005; revised December 16, 2005; accepted for publication December 21, 2005.

Available online February 21, 2006.

Reprint requests: Jamila Chakir, PhD, Centre de recherche, Hôpital Laval, 2725 Chemin Sainte-Foy, Sainte-Foy, Quebec, Canada, G1V 4G5.
E-mail: jamila.chakir@med.ulaval.ca.

0091-6749/\$32.00

© 2006 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.12.1349

Abbreviation used

HMC-1: Human mast cell

shown to increase collagen mRNA expression in skin fibroblasts.¹⁶ Our recent data provided evidence that IL-4, which is produced by T cells as well as mast cells, increased production of procollagen I (α_1) by bronchial fibroblasts derived from bronchial biopsies of subjects with asthma.¹⁷

IL-4 and IL-13 have also been shown to induce the production of cytokines (IL-6, IL-11, and GM-CSF) by pulmonary fibroblast cell lines.¹⁸ Moreover, IL-4 promotes the differentiation of synovial fibroblasts to myofibroblasts.¹⁹ The fibrogenic role of IL-4 has been documented in the skin.²⁰ IL-4 increases collagen and fibronectin synthesis by synovial fibroblasts.²¹ IL-4 mRNA can be alternatively spliced into IL-4 δ 2 variant. In this variant, exon 2 of the human IL-4 gene is omitted.²² This variant is expressed in much higher levels than IL-4 mRNA in thymocytes and bronchoalveolar lavage cells.²³ IL-4 δ 2 encodes a truncated protein that acts as a receptor antagonist of IL-4 and interferes with the activation of cells by IL-4.^{24,25} The IL-4 signal is conferred to effector cells through binding to the IL-4 receptors. These represent transmembrane receptors composed of IL-R α , the common γ chain, and IL-13R α 1.²⁶

Here, we explored the possibility that mast cells influence collagen production by bronchial fibroblasts derived from bronchial biopsies of subjects with asthma and normal subjects. We found that mast cells induced a significant increase in procollagen I (α_1) gene expression by fibroblasts from subjects with asthma, but not from normal controls. This effect is mediated by IL-4 because neutralization of IL-4 abrogates the collagen mRNA expression. IL-4/IL-4 δ 2 ratio is higher when mast cells were cultured with fibroblasts obtained from subjects with asthma compared with fibroblasts from healthy controls. Moreover, we found that fibroblasts from subjects with asthma expressed less IL-13R α 2 than cells from normal controls. This expression was not changed by coculture with mast cells.

METHODS

Cell cultures

The human mast cell line, HMC-1 was cultured in 75 cm² flasks in Iscove Dulbecco Eagle medium (Flow Laboratories, Mississauga, Ontario, Canada) supplemented with 10% FBS, 1% HEPES, and antibiotics.

Primary fibroblasts were isolated from bronchial biopsies of subjects with asthma ($n = 8$) and healthy ($n = 8$) subjects. The subjects with asthma fulfilling the American Thoracic Society criteria for asthma²⁷ were recruited from the Asthma Clinic at Laval Hospital. They used only an inhaled β_2 -agonist on demand. None used inhaled or systemic corticosteroids. All patients with asthma were atopic non-smokers (mean age, 28 years). Healthy subjects (mean age, 29 years) were nonatopic nonsmokers with no history of asthma or systemic diseases.²⁸ Bronchial biopsies were obtained by bronchoscopy

from subjects with asthma and healthy subjects. The study was approved by the ethics Committee at the Laval Hospital, and written informed consent was obtained from all subjects before entry into the study.

Isolated fibroblasts were characterized by immunofluorescence and flow cytometry using a mouse antivimentin and a mouse anti-human fibroblast antigen Ab-1 antibody (Calbiochem, San Diego, Calif) that shows no cross-reactivity with epithelial cells, endothelial cells, smooth muscle cells, or other cell types. This identification confirmed the purity of bronchial fibroblast cell culture as described previously.^{29,30}

Cocultures

Bronchial fibroblasts from healthy or subjects with asthma were plated at 2×10^5 cells/well in 6-well plates (Costar, Big Flats, NY) in 10% FBS supplemented Dulbecco modified Eagle medium and allowed to adhere overnight. The next day, medium was changed and mast cells, HMC-1, were added (2×10^5 cells/well) to the upper well of a trans-well system (Falcon, Franklin Lakes, NJ) for 4, 24, 48, and 72 hours. Supernatants were harvested and frozen at -80°C .

RNA extraction and real-time PCR

Total RNA was extracted from fibroblasts and HMC-1 after each coculture using the RNeasy Mini Kit (Qiagen, Mississauga, Ontario, Canada) following the manufacturer's protocol. Concentrations of total RNA were measured by a fluorescent dye with the Ribogreen RNA quantification reagent (Molecular Probes Inc, Eugene, Ore). Quantification of the mRNA message coding for β_2 -microglobulin, procollagen I (α_1) mRNA, and total form of IL-4 was performed by using Lightcycler technology (Roche Diagnostics, Mannheim, Germany). Quantification of mRNA of IL-4 isoforms (IL-4 and IL-4 δ 2), IL-4R α , IL-13R α 1, IL-13R α 2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed by using Smart-Cycler technology (Cepheid, Sunnyvale, Calif) as previously described.³¹ Primers used are listed in Table 1.³² PCR reactions were performed in a volume of 20 μL containing 1 μL cDNA, 0.3 $\mu\text{mol/L}$ of each primer, and 10 μL QuantiTect SYBR Green PCR (Qiagen, Mississauga, Ontario, Canada) containing Hot Start DNA polymerase, dNTP mix, buffer, MgCl_2 , and fluorescent dyes. The PCR protocol consisted of 3 programs: denaturation, amplification, and melting curve analysis for product identification. The denaturation and amplification conditions for β_2 -microglobulin were 95°C for 15 minutes followed by 45 cycles of PCR. Each cycle included denaturation at 95°C for 20 seconds, annealing of 20 seconds at 57°C , and extension of 20 seconds at 72°C . The temperature transition rate was 20°C/s , except when heating at 72°C , when it was at 5°C/s . Fluorescence was measured at the end of every cycle to allow quantification of cDNA. After amplification, a melting curve was obtained by slowly heating at 95°C with fluorescence detection every 0.2°C following normal cycle. With the use of standard curves, the amount of copy numbers of procollagen, IL-4, and IL-13R α 2 genes in each sample was calculated and normalized to β_2 -microglobulin or GAPDH in the same sample we previously reported.¹⁷

Cytokine measurements

Measurement of hIL-4 and IL-13 in culture supernatant samples was performed by using ELISA (R&D Systems, Minneapolis, Minn). The minimum detectable level of IL-4 was less than 0.13 pg/mL and 32 pg/mL for IL-13.

Neutralization

Neutralization of IL-4 was performed during cocultures. IL-4 neutralizing antibody ($1.25 \mu\text{g}/10^5$ cells; Pharmingen, San Diego, Calif) was added to fibroblast and mast cell cocultures. Cells were

TABLE I. Sequences and predicted sizes of primers used for PCR analysis

Molecules	Primers	Predicted size of amplicon
Collα1	5'-TGA ATC TGG ACG TGA GGG GG-3' sense 5'-CACGGTCACGAACCACAT TGG CAT-3' antisense	647 bp
IL-4 outer primers	5'-CGA GTT GAC CGT AAC AGA CAT-3' sense 5'-CGT CTT TAG CCT TTC CAA GAA G-3' antisense	281 bp
IL-4δ2 outer primers	5'-CAG AGC AGA AGA ACA CAA CTG-3' sense 5'-CGT CTT TAG CCT TTC CAA GAA G-3' antisense	254 bp
IL-4 and IL-4δ2 inner primers	5'-TGT GCT CCG GCA GTT CTA CAG-3' sense 5'-TGG CTT CCT TCA CAG GAC AGG-3' antisense	164 bp
IL-13 Rα2	5'-TTT CGT TTG CTT GGC TAT CG-3' sense 5'-TGT AAT GCA TGA TCC AAG CC-3' antisense	542 bp
GAPDH	5'-ATG CAA CGG ATT TGG TCG TAT-3' sense 5'-TCT CGC TCC TGG AAG ATG GTG-3' antisense	221 bp
β ₂ -Microglobulin	5'-GATGAGTATGCTGCCGTGTG-3' sense 5'-CAATCCAAATGCGGCATCT-3' antisense	115 bp

then washed and fibroblast RNA extracted, and RT-PCR for procollagen I (α₁) was performed as described.

Measurement of procollagen I protein production in culture supernatant

Procollagen I was measured with a radioimmunoassay kit (Pharmacia and Upjohn, Don Mills, Ontario, Canada) as we previously reported.²⁹

Statistical analysis

All experiments in this study were performed at least 3 times. The data shown are representative results. Experimental values are given as means ± SDs. To compare groups and conditions, the data were analyzed by using a crossed-nested design. When interactions between groups and conditions were significant, each group was analyzed by using a randomized block design. A posteriori multiple comparisons were performed by using Tukey-Kramer test. Mean values ± SEMs of quantitative variables were used as representative measures. All reported *P* values were 2-sided and were declared significant at .05 levels.

RESULTS

Procollagen I (α₁) gene and protein expression by fibroblasts

A time course analysis was performed to evaluate procollagen I (α₁) gene expression by fibroblasts in the presence or absence of mast cells. At baseline, fibroblasts derived from subjects with asthma and healthy subjects expressed the same level of procollagen I (α₁) gene (Fig 1). This expression did not change over time of incubation. Coculture with mast cells induced a significant increase in the procollagen I (α₁)/β₂ microglobulin ratio in fibroblasts derived from subjects with asthma after 72 hours of culture (*P* = .04; Fig 1, A). However, there was no significant change of procollagen I (α₁) gene expression in normal control-derived fibroblasts (Fig 1, B).

Measurement at protein level showed that procollagen protein expression was significantly increased in asthmatic cells when cocultured with mast cells (from 43.04 ± 6 to 73 ± 5 μg/mL; *P* = .017). There was no significant change in the normal cells (from 41 ± 6 to 33 ± 5 μg/mL).

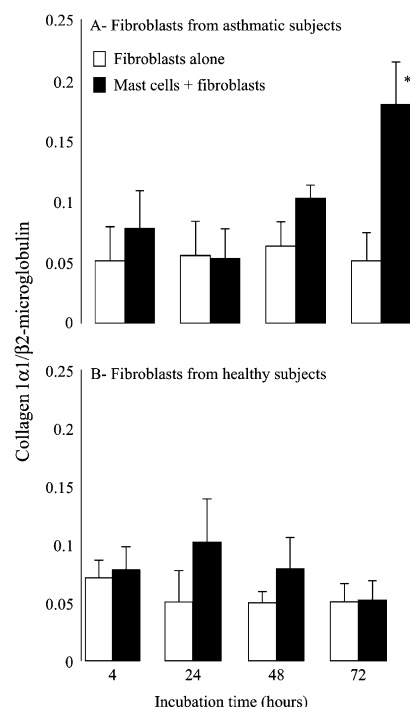


FIG 1. Procollagen 1 (α₁) gene expression by bronchial fibroblast derived from subjects with asthma (A) and normal controls (B) measured by real-time RT-PCR. Each value represents the average ± SEM (n = 5). **P* = .04.

IL-4 and IL-4δ2 gene expression by mast cells

To determine whether fibroblasts could modulate cytokine production in mast cells, IL-4 gene expression was investigated. IL-4δ2-gene expression by mast cells as measured by real-time PCR did not change over time in coculture with fibroblasts from subjects with asthma or from normal subjects (Fig 2, A). However, total IL-4 gene expression was significantly increased 48 (*P* = .019) and 72 hours (*P* = .012) after coculture of mast cells with fibroblasts from subjects with asthma compared with mast cells

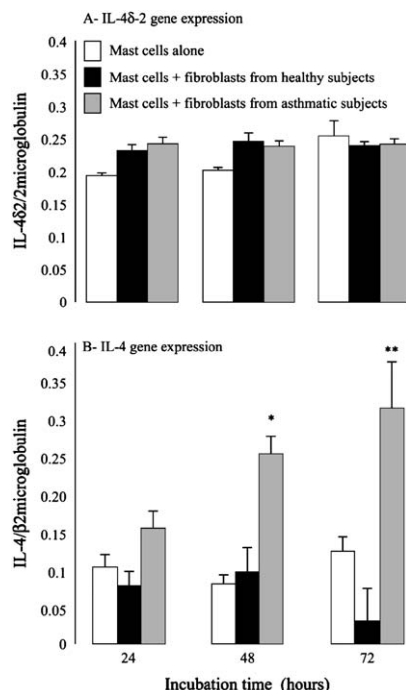


FIG 2. IL-4δ2 (A) and IL-4 (B) gene expressions by mast cells. IL-4 and IL-4δ2 gene expression was measured by real-time RT-PCR. Each value represents the average $\pm$ SEM (n = 5). * P = .01; ** P = .01.

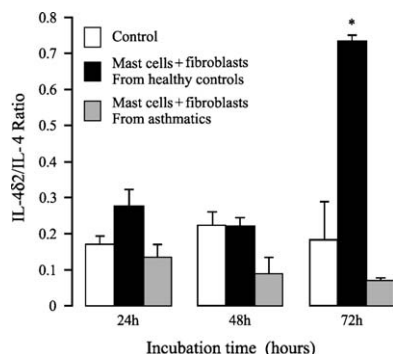


FIG 3. IL-4 production by mast cells cocultured with asthmatic fibroblasts. Each value represents the average $\pm$ SEM (n = 8). * P = .03.

alone or cultured with fibroblasts from normal controls (Fig 2, B).

Fig 3 shows IL-4δ2 /IL-4 ratio. This ratio is significantly higher at 72 hours (P = .001) when mast cells were cocultured with fibroblasts derived from healthy controls compared with fibroblasts from subjects with asthma.

IL-4 and IL-13 production by mast cells

We further investigated IL-4 and IL-13 production at the protein level. Total IL-4 produced by mast cells cocultured with bronchial fibroblasts from asthmatic and healthy subjects for 24, 48, and 72 hours was measured in cell free supernatants. There was no significant change in IL-4 production by mast cells when they were cocultured

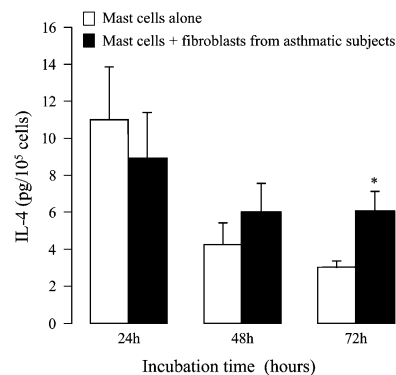


FIG 4. IL-4δ2/IL-4 ratio. Each value represents the average $\pm$ SEM (n = 5). * P = .001.

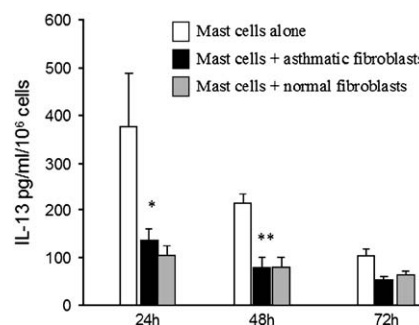


FIG 5. IL-13 production by mast cells cultured alone or in presence of fibroblasts obtained from subjects with asthma or normal subjects. * P = .02; ** P = .01.

with normal fibroblasts (data not shown). However, when they were cocultured with asthmatic fibroblasts, IL-4 production was significantly increased (P = .03) at 72 hours compared with control conditions (Fig 4). We also measured IL-13 production. There was no significant change in IL-13 release when mast cells were cultured with asthmatic and normal cells. However, coculture decreased baseline production of IL-13 by mast cells by about 56% (Fig 5).

Neutralization

To evaluate whether the increase in procollagen gene expression is mediated by IL-4, we added an IL-4 neutralizing antibody to the coculture. Fig 6 shows that neutralization of IL-4 abrogates the increase of procollagen gene expression in fibroblasts derived from asthmatic subjects, suggesting an important role of IL-4 in the modulation of procollagen production.

IL-4Rα, IL-13Rα1, and IL-13RαII expression

We looked at the expression of IL-4 and IL-13 receptors on bronchial fibroblasts at baseline and after coculture with mast cells. There was no significant difference in IL-4Rα and IL-13Rα1 expression in fibroblasts from subjects with asthma compared with those from healthy controls at baseline and after coculture with mast cells (data not shown). However, IL-13RαII, a decoy receptor,

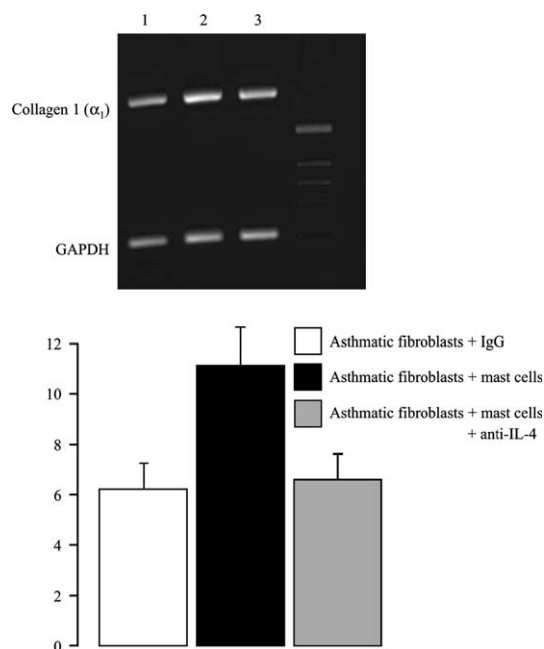


FIG 6. Neutralization tests. *Top* represents 1 representative RT-PCR experiment. *Lane 1*, Asthmatic fibroblasts + IgG; *lane 2*, asthmatic fibroblasts + mast cells; *lane 3*, asthmatic fibroblasts + mast cells + anti-IL-4. *Bottom* shows the relative intensities of bands. Experiments were repeated at least 3 times ($n = 3$).

was significantly ($P = .02$) decreased in asthmatic fibroblasts compared with normal fibroblasts (Fig 7). Coculture conditions did not change the baseline level of IL-13-R α II expression in normal and asthmatic fibroblasts.

DISCUSSION

There is growing evidence that interplay between inflammatory and structural cells plays an important role in the physiopathology of asthma. In this study, we presented evidence that mast cells induce procollagen gene expression by bronchial fibroblasts from subjects with asthma through IL-4 production, thus providing a regulatory loop for amplification of inflammation in asthma. We also showed for the first time that mast cells express IL-4 δ 2 isoform, an alternative splice variant of human IL-4. IL-4 δ 2 isoform acts as an IL-4 antagonist and thus inhibits its effect by binding to IL-4 receptors.²⁵ Interestingly, IL-4 and IL-4 δ 2 gene expression was dependent on the nature of fibroblasts cultured with mast cells. Thus, the overall IL-4 δ 2/IL-4 ratio is higher when mast cells were cocultured with fibroblasts derived from healthy controls compared with fibroblasts from subjects with asthma. This suggests that possibly, in normal conditions, bronchial fibroblasts may stimulate preferentially IL-4 δ 2 gene expression rather than IL-4. Consequently, procollagen I (α_1) gene is not induced in these cells. However, actually it is not possible to measure IL-4 δ 2 protein levels. The activity of IL-4 has not been distinguished from that of IL-4 δ 2 because validated protein assays that specifically

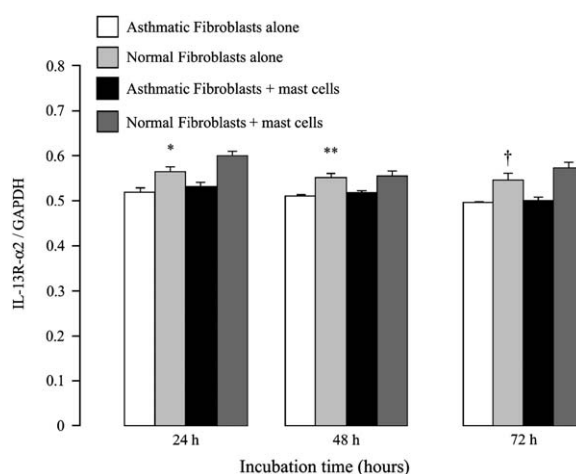


FIG 7. Real-time PCR measurement of IL-13R α 2 gene in fibroblasts derived from subjects with asthma and healthy subjects cultured alone or in the presence of mast cells. Each value represents the average $\pm$ SEM ($n = 8$).

detect IL-4 δ 2 have yet to be developed. IL-4 δ 2 gene expression has been described in asthma.³³ Seah et al³⁴ have shown that IL-4 δ 2 gene expression is lower in patients with asthma compared with control subjects. Hence, the ratio of IL-4 to IL-4 δ 2 may be important in the regulation of IL-4 effect in asthma. Atamas et al³⁵ have reported that IL-4 δ 2 induces collagen I (α_2) gene expression by skin and lung fibroblasts, and in this case, it seems to synergize with IL-4. They suggest that the action of IL-4 δ 2 is dependent on its ability to bind to different IL-4 receptors. The activity of IL-4 has not been distinguished from that of IL-4 δ 2 because validated protein assays that specifically detect IL-4 δ 2 have yet to be developed. IL-13 is produced by mast cells, and coculture with fibroblasts from both subjects with asthma and healthy controls decreased this production at the same level. This may be a result of different factors produced by bronchial fibroblasts such as TGF- β . Thus, it has been shown recently that TGF- β inhibits growth, maturation, granule formation, and mediator release in mast cells.^{36,37} Collagen deposition underneath the basement membrane is a hallmark of asthma. This seems to be a result of the activation of subepithelial fibroblasts. These cells acquire the myofibroblast phenotype. There is a correlation between the number of myofibroblasts in the bronchial mucosa and the degree of subepithelial fibrosis observed in asthma.¹³ These fibroblasts increase in number after an allergen challenge.³⁸ We have shown that in asthma mesenchymal cells undergo functional and phenotypic alterations, which they keep after *in vitro* culture and which may play a key role in the manifestation of the disease.^{29,39,40} It is largely believed that airway remodeling results from persistent inflammation, the repair process, and recurrent physical stresses. Thus, we found that T_H2-type cytokines, particularly IL-4, have a direct regulatory effect on collagen metabolism by increasing collagen production and decreasing its degradation. This effect is higher with fibroblasts derived from subjects with

asthma.⁴¹ In the current study, we show that this effect can also be indirect through interactions with inflammatory cells such as mast cells, suggesting that, in asthma, fibroblasts can not only respond to T_H2 cytokines but also induce release of these cytokines by inflammatory cells. This release seems tightly regulated by the activation state of bronchial fibroblasts. Interestingly enough, fibroblasts from normal controls promote the expression of the splice variant of IL-4 that is known for its inhibitory effect of IL-4. In asthma, the ratio is in favor of IL-4, thus facilitating its effect on bronchial fibroblasts.

IL-4 signals through receptor complexes. Type I IL-4R consists of IL-4R α and the common γ chain, and this receptor predominates in hematopoietic cells.²⁶ IL-4R α and IL-13R α 1 form the type II receptor, a receptor shared by both IL-4 and IL-13.⁴² Hematopoietic and nonhematopoietic cells express this receptor.⁴³ Another IL-13-binding chain, IL-13R α 2, exists in a monomeric form and binds to IL-13 with high affinity.⁴⁴ IL-13R α 2 appears to act as a decoy receptor that inhibits the activity of IL-13.⁴⁵ IL-13R α 2 expression is upregulated by IL-4 and IL-13 in bronchial epithelial cells and thus downregulated the IL-13 signal in these cells.⁴⁶ In the current work, we found that fibroblasts derived from subjects with asthma express low level of IL-13R α 2 compared with fibroblasts derived from healthy controls. This suggests that the effect of IL-13 is facilitated in the asthmatic cells.

Interaction between mast cells and fibroblasts has been well documented, particularly in the skin. Mast cells induce cultured fibroblasts to proliferate and synthesise increased amounts of collagen.^{47,48} They produce an array of mediators that have been shown to stimulate fibroblasts. For example, histamine increases procollagen I production by keloid fibroblasts,⁴⁹ and tryptase, a specific mast cell neutral protease, has been also reported to induce proliferation and collagen gene expression in human skin fibroblasts.⁸ In our conditions, neutralization of IL-4 abrogates completely procollagen I (α ₁) gene expression, indicating that the observed increase in this gene is mainly a result of IL-4. Our experiments cannot exclude, however, that IL-4 can have an indirect effect and acts through other mediators.

In conclusion, we showed that mast cells can influence the function of bronchial fibroblasts derived from subjects with asthma and that IL-4 and IL-4 δ 2 may play an important role in collagen regulation in asthma.

We are grateful to Luce Trépanier for subject recruitment and evaluation, Véronique Turmel for her technical help, and Serge Simard for statistical analysis.

REFERENCES

- McCurdy JD, Olynych TJ, Maher LH, Marshall JS. Cutting edge: distinct toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells. *J Immunol* 2003;170:1625-9.
- Yamamoto T, Hartmann K, Eckes B, Krieg T. Role of stem cell factor and monocyte chemoattractant protein-1 in the interaction between fibroblasts and mast cells in fibrosis. *J Dermatol Sci* 2001;26:106-11.

- Mekori YA. The mastocyte: the "other" inflammatory cell in immunopathogenesis. *J Allergy Clin Immunol* 2004;114:52-7.
- Bradding P. The role of the mast cell in asthma: a reassessment. *Curr Opin Allergy Clin Immunol* 2003;3:45-50.
- Jordana M. Mast cells and fibrosis: who's on first? *Am J Respir Cell Mol Biol* 1993;8:7-8.
- Gruber BL. Mast cells in the pathogenesis of fibrosis. *Curr Rheumatol Rep* 2003;5:147-53.
- Liebler JM, Qu Z, Buckner B, Powers MR, Rosenbaum JT. Fibroproliferation and mast cells in the acute respiratory distress syndrome. *Thorax* 1998;53:823-9.
- Garbuzenko E, Nagler A, Pickholtz D, Gillery P, Reich R, Maquart FX, et al. Human mast cells stimulate fibroblast proliferation, collagen synthesis and lattice contraction: a direct role for mast cells in skin fibrosis. *Clin Exp Allergy* 2002;32:237-46.
- Berton A, Levi-Schaffer F, Emonard H, Garbuzenko E, Gillery P, Maquart FX. Activation of fibroblasts in collagen lattices by mast cell extract: a model of fibrosis. *Clin Exp Allergy* 2000;30:485-92.
- Reed JA, Albino AP, McNutt NS. Human cutaneous mast cells express basic fibroblast growth factor. *Lab Invest* 1995;72:215-22.
- Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520-4.
- Chakir J, Laviolette M, Boutet M, Laliberté R, Dubé J, Boulet L-P. Lower airways remodeling in nonasthmatic subjects with allergic rhinitis. *Lab Invest* 1996;75:735-44.
- Brewster CEP, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990;3:507-11.
- Bradding P, Okayama Y, Howarth PH, Church MK, Holgate ST. Heterogeneity of human mast cells based on cytokine content. *J Immunol* 1995;155:297-307.
- Holgate ST. The mast cell and its function in allergic disease. *Clin Exp Allergy* 1991;21(suppl 3):11-6.
- Gruber BL, Kew RR, Jelaska A, Marchese MJ, Garlick J, Ren S, et al. Human mast cells activate fibroblasts: tryptase is a fibrogenic factor stimulating collagen messenger ribonucleic acid synthesis and fibroblast chemotaxis. *J Immunol* 1997;158:2310-7.
- Bergeron C, Page N, Joubert P, Barbeau B, Hamid Q, Chakir J. Regulation of procollagen I (α 1) by interleukin-4 in human bronchial fibroblasts: a possible role in airway remodelling in asthma. *Clin Exp Allergy* 2003;33:1389-97.
- Doucet C, Brouty-Boyd D, Pottin-Clémenceau 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.
- Mattey DL, Dawes PT, Nixon NB, Slater H. Transforming growth factor beta 1 and interleukin 4 induced alpha smooth muscle actin expression and myofibroblast-like differentiation in human synovial fibroblasts in vitro: modulation by basic fibroblast growth factor. *Ann Rheum Dis* 1997;56:426-31.
- Fertin C, Nicolas JF, Gillery P, Kalis B, Banchereau J, Maquart FX. Interleukin-4 stimulates collagen synthesis by normal and scleroderma fibroblasts in dermal equivalents. *Cell Mol Biol* 1991;37:823-9.
- Postlethwaite AE, Holness MA, Katai H, Raghow R. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. *J Clin Invest* 1992;90:1479-85.
- Alms WJ, Atamas SP, Yurovsky VV, White B. Generation of a variant of human interleukin-4 by alternative splicing. *Mol Immunol* 1996;33:361-70.
- Atamas SP, Choi J, Yurovsky VV, White B. An alternative splice variant of human IL-4, IL-4 delta 2, inhibits IL-4-stimulated T cell proliferation. *J Immunol* 1996;156:435-41.
- Asokanathan N, Graham PT, Stewart DJ, Bakker AJ, Eidne KA, Thompson PJ, et al. House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J Immunol* 2002;169:4572-8.
- Arinobu Y, Atamas SP, Otsuka T, Niino H, Yamaoka K, Mitsuyasu H, et al. Antagonistic effects of an alternative splice variant of human IL-4, IL-4 δ 2, on IL-4 activities in human monocytes and B cells. *Cell Immunol* 1999;191:161-7.
- Keegan AD, Nelms K, White M, Wang LM, Pierce JH, Paul WE. An IL-4 receptor region containing an insulin receptor motif is important

- for IL-4-mediated IRS-1 phosphorylation and cell growth. *Cell* 1994;76: 811-20.
27. Standardization of spirometry: 1987 update. Statement of the American Thoracic Society. *Am Rev Respir Dis* 1987;136:1285-98.
28. Goulet F, Boulet L-P, Chakir J, Tremblay N, Dubé J, Laviolette M, et al. Morphologic and functional properties of bronchial cells isolated from normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 1996;15: 312-8.
29. Dubé J, Chakir J, Laviolette M, Saint-Martin S, Boutet M, Desrochers C, et al. In vitro procollagen synthesis and proliferative phenotype of bronchial fibroblasts from normal and asthmatic subjects. *Lab Invest* 1998;78: 297-307.
30. Chakir J, Dubé J, Laviolette M, Goulet F, Germain L, Auger F, et al. Isolation and characterization of human airway fibroblasts in culture. In: Chung KF, Adcock IM, editors. *Methods in molecular medicine: asthma*. Totowa (NJ): Humana Press; 1999. p. 53-65.
31. Prieto J, Van Der Ploeg I, Roquet A, Gigliotti D, Dahlen B, Eklund A, et al. Cytokine mRNA expression in patients with mild allergic asthma following low dose or cumulative dose allergen provocation. *Clin Exp Allergy* 2001;31:791-800.
32. Seah GT, Rook GAW. A sensitive, non-radioactive quantitative method for measuring IL-4 and IL-482 mRNA in unstimulated cells from multiple clinical samples, using nested RT-PCR. *J Immunol Methods* 1999; 228:139-49.
33. Glare EM, Divjak M, Rolland JM, Walters EH. Asthmatic airway biopsy specimens are more likely to express the IL-4 alternative splice variant IL-482. *J Allergy Clin Immunol* 1999;104:978-82.
34. Seah GT, Gao PS, Hopkin JM, Rook GAW. Interleukin-4 and its alternatively spliced variant (IL-482) in patients with atopic asthma. *Am J Respir Crit Care Med* 2001;164:1016-8.
35. Atamas SP, Yurovsky VV, Wise R, Wigley FM, Goter Robinson CJ, Henry P, et al. Production of type 2 cytokines by CD8+ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. *Arthritis Rheum* 1999;42:1168-78.
36. Gebhardt T, Lorentz A, Detmer F, Trautwein C, Bektas H, Manns MP, et al. Growth, phenotype, and function of human intestinal mast cells are tightly regulated by transforming growth factor beta1. *Gut* 2005;54: 928-34.
37. Kashyap M, Bailey DP, Gomez G, Rivera J, Huff TF, Ryan JJ. TGF-beta(1) inhibits late-stage mast cell maturation. *Exp Hematol* 2005;33: 1281-91.
38. Gizycki MJ, Adelroth E, Rogers AV, O'Byrne PM, Jeffery PK. Myofibroblast involvement in the allergen-induced late response in mild atopic asthma. *Am J Respir Cell Mol Biol* 1997;16:664-73.
39. Laliberté R, Rouabhia M, Bossé M, Chakir J. Decreased capacity of asthmatic bronchial fibroblasts to degrade collagen. *Matrix Biol* 2001;19: 743-53.
40. Dubé J, Chakir J, Dubé C, Grimard Y, Laviolette M, Boulet L-P. Synergistic action of endothelin (ET)-1 on the activation of bronchial fibroblast isolated from normal and asthmatic subjects. *Int J Exp Pathol* 2000;81: 429-37.
41. Bergeron C, Pagé N, Joubert P, Barbeau B, Hamid Q, Chakir J. Regulation of procollagen I (α 1) by interleukin-4 in human bronchial fibroblasts: a possible role in airway remodelling in asthma. *Clin Exp Allergy* 2003;33:1389-97.
42. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol* 1999;17:701-38.
43. Doucet C, Giron-Michel J, Canonica GW, Azzarone B. Human lung myofibroblasts as effectors of the inflammatory process: the common receptor γ chain is induced by Th2 cytokines, and CD40 ligand is induced by lipopolysaccharide, thrombin and TNF- α . *Eur J Immunol* 2002;32: 2437-49.
44. David MD, Bertoglio J, Pierre J. Functional characterization of IL-13 receptor alpha2 gene promoter: a critical role of the transcription factor STAT6 for regulated expression. *Oncogene* 2003;22:3386-94.
45. Mentink-Kane MM, Wynn TA. Opposing roles for IL-13 and IL-13 receptor alpha 2 in health and disease. *Immunol Rev* 2004;202: 191-202.
46. Yoshikawa M, Nakajima T, Tsukidate T, Matsumoto K, Iida M, Otori N, et al. TNF-alpha and IL-4 regulate expression of IL-13 receptor alpha2 on human fibroblasts. *Biochem Biophys Res Commun* 2003;312: 1248-55.
47. Abe M, Kurosawa M, Ishikawa O, Miyachi Y. Effect of mast cell-derived mediators and mast cell-related neutral proteases on human dermal fibroblast proliferation and type I collagen production. *J Allergy Clin Immunol* 2000;106:S78-84.
48. Trautmann A, Krohne G, Bröcker E-B, Klein CE. Human mast cells augment fibroblast proliferation by heterotypic cell-cell adhesion and action of IL-4. *J Immunol* 1998;160:5053-7.
49. Kikuchi K, Kadono T, Takehara K. Effects of various growth factors and histamine on cultured keloid fibroblasts. *Dermatology* 1995;190:4-8.