

ORIGINAL PAPER

Effect of glucocorticoids on stem cell factor expression in human asthmatic bronchi

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Clinical and
Experimental
Allergy

Summary

Background Stem cell factor (SCF) is a major mast cell growth factor promoting differentiation, chemotaxis as well as inhibition of apoptosis of mast cells. Regulation of SCF expression by glucocorticoids has not yet been reported in human asthmatic bronchi.

Objective To evaluate SCF mRNA and protein expression in biopsy specimen and bronchoalveolar lavage fluid, respectively, and to determine the mast cell numbers in biopsy sections from control and asthmatic subjects treated or not with glucocorticoids.

Methods Volunteers were recruited out of pollen season. Asthmatic patients were allergic to common allergen extracts including grass and tree pollen, cat, dog or mite; three volunteers had non-allergic asthma. Mast cell numbers were counted after anti-human tryptase immunolabelling. SCF mRNA was quantified by real-time fluorescent PCR (LightCycler™) after reverse transcription, and SCF protein was measured by ELISA.

Results Asthmatic patients not treated with glucocorticoids showed a 5.8-, 1.8- and 3.1-fold significant increase in SCF mRNA, protein levels and mast cell numbers, respectively, compared with healthy volunteers of the control group (7.8 and 1.3 pg/μg SCF mRNA/GAPDH; 99.8 ± 11.5 and 56.0 ± 11.0 pg/mL SCF protein; 103 ± 21 and 33 ± 8 mast cells/mm², respectively; $P < 0.05$). In asthmatic patients treated with glucocorticoids, a significant decrease of SCF mRNA, protein levels and mast cell numbers was observed as compared with untreated asthmatic patients (1.1 pg/μg mRNA; 62.0 ± 10.3 pg/mL SCF protein and 39 ± 13 mast cells/mm²; $P < 0.05$), reaching levels comparable to those of the control group.

Conclusion Our study shows that SCF is expressed in the bronchus in humans *in vivo*. This expression is increased in asthma, and is parallel to the increased mast cell numbers in the airways. Both increases were normalized in glucocorticoid-treated patients, strongly suggesting an involvement of SCF in the mast cell-associated asthmatic disease.

Keywords airways, asthma, glucocorticoids, human, mast cell, SCF

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Introduction

Stem cell factor (SCF), also termed Kit ligand, steel factor or mast cell growth factor [1–3], acts as an important growth factor for human mast cells [4], inducing *in vitro* the proliferation and differentiation of immature mast cells from bone marrow CD34⁺ progenitor cells [5]. SCF is also involved in mast cell survival by inhibition of their apoptosis [6]. SCF is a potent chemotactic factor for the mast cells [7], and promotes their adhesion to the extracellular matrix [8]. In addition, SCF induces a mast cell

hyperplasia *in vivo* after sub-cutaneous injection in humans [9]; mast cell hyperplasia observed in systemic mastocytosis is associated with enhanced SCF expression [10]. Finally, SCF considerably enhances *in vitro* the antigen-induced degranulation of human lung-derived mast cells [11], and induces by itself mast cell degranulation *in vitro* [12] and *in vivo* [9]. The expression of SCF might therefore be enhanced in diseases associated with an increased number and activation of mast cells like asthma.

The increase in mast cell numbers is observed in human asthmatic airways even in the absence of allergen

exposure as compared with non-asthmatic patients [13, 14]. These inflammatory cells are mainly infiltrated in the bronchial mucosa, particularly within the bronchial epithelium [13, 15]. After allergen exposure, mast cell numbers are further increased in the bronchial epithelium of asthmatic patients [16, 17] compared with healthy subjects. In parallel, SCF expression is increased within the human asthmatic airways [18], and in a murine model of asthma, allergen exposure leads to increased SCF expression in the lung and serum [19]. Patients with seasonal rhinitis also exhibit increased SCF expression in nasal ciliated epithelial cells [20], which is correlated with the increased mast cell number [21].

In the lower airways, SCF may be produced by various cells, as it is the case *in vivo* by human bronchial epithelial cells [18], and *in vitro* by bronchial epithelial cells in culture [22], bronchial subepithelial myofibroblasts [23], lung fibroblasts [24], airway smooth muscle cells [25] and the isolated human lung mast cells themselves [26, 27]. SCF is also produced by cultured endothelial cells of other origins [28]. Many of these cell types are important participants in the inflammatory condition of the asthmatic airways.

We have previously reported that, *in vitro*, the expression of SCF is increased under inflammatory conditions and decreased by glucocorticoid treatment [24]. However, comparison of SCF production in the human asthmatic bronchi during treatment by glucocorticoids has not yet been reported. Therefore, the aim of the present study was to evaluate the expression of SCF locally in the airways and compare it between control subjects and asthmatic patients treated or not with glucocorticoids. As glucocorticoids are reported to decrease mast cell numbers and activation in the airways [29, 30], we have verified whether infiltrated mast cell numbers varied between asthmatic patients treated or not with glucocorticoids and control subjects.

Material and methods

Subjects

Fourteen asthmatic patients (seven males, seven females, mean age: 32.9 ± 3.3 years) with clinically stable intermittent or persistent mild to moderate bronchial asthma (as defined by the American Thoracic Society, International Consensus, 1992) were recruited and categorized into two groups according to the medication required to control their symptoms. One group (A^+G^- ; $n = 7$) comprised of asthmatic patients not treated by glucocorticoids and occasionally treated by inhaled β_2 -bronchodilator. The other group (A^+G^+ ; $n = 7$) comprised of asthmatic patients treated by glucocorticoids, and bronchodilators (long-acting β_2 agonist and/or theophyllin) on a daily basis for at least 1 month prior to the study. All but one patient

from the A^+G^- group, and two patients from the A^+G^+ group were non-allergic, as indicated by the weal and flare responses to common allergen extracts including grass pollen, tree pollen, cat, dog and mite. Glucocorticoids were withheld 8 h before the bronchofibroscopy and bronchodilators 6 h before.

Control subjects (C ; $n = 7$) were all healthy non-atopic volunteers (three males, four females, mean age: 25.7 ± 1.9 years). They had no history of symptoms suggestive of asthma or rhinitis, and had negative skin prick tests to the above-mentioned common allergens. All of them had a negative methacholine challenge test, and no regular medication other than contraceptives for females.

Most of the subjects were non-smokers, and none of them had any respiratory infection during the 4 weeks preceding inclusion. The study was approved by the ethical committee of our hospital, and all patients gave their written informed consent. The clinical details of the 21 volunteers studied herein are summarized in Tables 1 and 2.

Study design

The recruited volunteers and patients came into the out-clinic of our department 5 days prior to bronchoscopy. A non-specific bronchial hyper-responsiveness test was measured as the provocative concentration of methacholine required to reduce the forced expiratory volume in 1 sec by 20% of the prechallenge baseline (PD_{20}). On the day of the study, subjects underwent a bronchoscopy procedure as described below (as per ATS guidelines).

Fibreoptic bronchoscopy

Fibreoptic bronchoscopy was performed according to our previously published protocol [31]. Briefly, after premedication of the subjects with diazepam (10 mg *per os*), a fibreoptic bronchoscope (Olympus BF P30, Paris, France) was introduced locally under 1% adrenaline-free xylocaine local anaesthesia. Three bronchial biopsies were taken from subsegmental divisions of the main bronchi in the left lower and upper lobes using alligator forceps. Bronchoalveolar lavage (BAL) was performed by 50 mL instillations of sterile apyrogenic isotonic saline in the lingual four times.

Bronchial biopsies and Bronchoalveolar lavage fluid processing

Two bronchial biopsies were gently extracted from the forceps, and immediately transferred into 400 μ L Tri Reagent[®] (Euromedex, Souffelweyersheim, France) for RNA extraction. Two biopsies were placed in 10% buffered formaldehyde fixative at 4 °C for 3 h. Subsequently, the

Table 1. Clinical characteristics of control subjects

Subject no.	Age	Gender	Smoking habits	Allergy/atopy	FEV ₁ (% pred)	PD ₂₀ (µg)	Treatment
1	38	M	O	No	90.3	> 2340	–
2	25	M	O	No	101.8	> 2340	–
3	21	F	C*	No	95.1	> 2340	–
4	28	M	C†	No	99.4	> 2340	–
5	26	F	No	No	103.6	> 2340	–
6	23	F	No	No	113.1	> 2340	–
7	24	F	No	No	102.2	> 2340	–

*10 cigarettes/day.

†5 cigarettes/day.

FEV₁, forced expiratory volume in one second; PD₂₀, provocation dose causing 20% fall in FEV₁; M, male; F, female; O, occasional smoker; C, current smoker.

biopsies were embedded in paraffin and kept until use for immunohistochemistry.

The recovered BAL fluid (BALF), with the exception of the first 20 mL recovered, was kept on ice and immediately transported to the laboratory. The fluid was strained through a single layer of sterile cotton gauze to remove mucus. The fluid phase was centrifuged at 400 *g* for 10 min and the cell-free BALF was then centrifuged at 1500 *g* for 15 min at 4 °C to remove debris, then aliquoted, lyophilized and kept frozen at – 80 °C.

RNA extraction and reverse transcription (RT)

Total RNA was isolated from the bronchial biopsies by two successive extractions using Tri Reagent® (1 mL) (Euro-

medex). Five micro litres of total RNA were incubated with 0.5 µg of random hexamers for 5 min at 70 °C in RNase-free water in a total volume of 10 µL, and allowed to cool down at room temperature. Total RNA was subsequently reverse transcribed in 1 × RT buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, pH 8.3) containing 1 mM of each deoxyribonucleotide triphosphate (dNTP), 1 U/µL RNasin ribonuclease inhibitor and 10 U/µL Moloney murine leukaemia virus reverse transcriptase (M-MLV RT) (all reagents provided by Promega, Madison, WI, USA), to a total volume of 20 µL. The reaction was conducted for 1 h at 37 °C, after what the reverse transcriptase was heat-inactivated at 99 °C for 5 min.

Quantification of stem cell factor messenger RNA

SCF and GAPDH (used as a domestic gene) cDNAs were amplified from cDNA obtained after reverse transcription by real-time fluorescent PCR (LightCycler™-SYBR Green I, Roche Diagnostics), with primers leading to a single 149 and 240 bp PCR products, respectively. PCR reactions were performed in 1 × PCR reaction buffer (2 µL of the reaction mix, containing FastStart Taq DNA polymerase, dNTP mix, SYBR Green I, 3 mM MgCl₂ (Roche Diagnostics)), and 10 pmol of each primer:

SCF amplification; sense primer: 5'-TGGATAAGCGAG ATGGTAGT-3' antisense primer: 5'-TTTTCTTTCACGCACT CCAC-3', *GAPDH amplification*; sense primer: 5'-GGTGA AGGTCGAGTCAACGGA-3' antisense primer: 5'-GAGG GATCTCGTCTCCTGGAAGA-3', in a 20 µL final volume for 35 cycles, with each cycle consisting of 15 s denaturation

Table 2. Clinical characteristics of asthmatic subjects

Subject no.	Age	Gender	Smoking habits	Allergy/atopy	FEV ₁ (% pred)	PD ₂₀ (µg)	Treatment
8	21	F	C*†	Cat, dog, grass pollen	93	36	–
9	25	M	No	Mite	83.8	72	–
10	22	M	No	Cat, mite	75.0	18	–
11	26	F	No	Cat	102.9	1620	–
12	24	F	C*†	Cat, grass pollen, mite	78.2	360	–
13	24	F	No	Non-allergic asthma	97.1	1620	–
14	22	M	No	Birch, mite, mould, food‡	75.0	1620	–
15	48	M	No	Mite, grass pollen	73.0	25	+
16	44	M	O	Non-allergic asthma	65.3	600	+
17	40	F	No	Mite, grass pollen	73.2	75	+
18	48	F	No	Non-allergic asthma	85.4	> 2340	+
19	50	M	No	Grass pollen	100.5	600	+
20	48	F	O	Mite, grass pollen, moulds	102	800	+
21	24	M	O	Mite, grass pollen	91.2	200	+

†5 cigarettes/day.

*10 cigarettes/day.

‡Coriander.

FEV₁, forced expiratory volume in one second; PD₂₀, provocation dose causing 20% fall in FEV₁; M, male; F, female; O, occasional smoker; C, current smoker.

at 95 °C, 10 s annealing at 53 °C or 60 °C for SCF and GAPDH cDNA amplification, respectively, and 10 sec extension at 72 °C. Amplified SCF and GAPDH cDNAs and a standard SCF and GAPDH cDNAs were analysed on-line by fluorescence (LightCycler™, Roche Diagnostics).

The standard SCF and GAPDH cDNAs were obtained from pulmonary fibroblast total cDNA. After amplification by PCR with the primers described above, the 149 bp- and 240 bp-PCR products were electrophoresed on a 2% agarose gel stained with ethidium bromide, purified on QIAEX II (QIAEX II gel extraction kit, QIAGEN, Courtaboeuf, France) and quantified by fluorescence (PicoGreen®, Molecular Probes Inc., Eugene, OR, USA) according to a standard curve obtained with a double-stranded phage λ DNA (0.005–1 µg/mL). This purified SCF and GAPDH cDNAs were used as a standard curve from 1 to 300 fg/mL. Quantification of SCF cDNA (fg) was normalized to GAPDH cDNA levels.

Stem cell factor enzyme-linked immunosorbent assay

The levels of SCF protein were quantified in bronchoalveolar lung fluid (BALF) from glucocorticoid-treated and untreated asthmatic patients and control subjects by a sensitive ELISA procedure, using a capture anti-human SCF monoclonal antibody (clone 13302.6; R&D Systems Europe, Abingdon, UK), and a biotinylated detection anti-human SCF polyclonal antibody (R&D Systems Europe), revealed by extravidin-horseradish peroxidase (Sigma Chemicals) and a 3,3',5,5'-tetramethylbenzidine liquid substrate system (Sigma Chemicals). Standard curves were generated with recombinant human SCF (R&D Systems Europe) diluted in NaCl 0.9%, and were linear from 3.9 to 500 pg/mL.

Mast cells counts

Adjacent 4 µm sections of paraffin-embedded bronchial biopsy specimen were cut and used for immunohistochemistry. Briefly, sections were deparaffinized in xylene and rehydrated through graded concentrations of ethanol. Samples were then incubated for 90 min at room temperature with a mouse monoclonal anti-human-tryptase antibody (0.25 µg/mL, Novacastra, Peterborough, UK). Staining was revealed by an anti-mouse secondary antibody (Rockland, Gilbertsville, PA, USA), coupled with the Vector® blue incubated with the FastBlue substrate, according to the manufacturer's instructions for the use of the alkaline phosphatase Vector® blue Kit (Vector Laboratories Inc., Burlingame, CA, USA). Tissue sections were counterstained with nuclear red and mounted with Glycergel medium (Dako Corp., Carpinteria, CA, USA). Negative controls were prepared by omitting the primary antibody. Results are expressed as the number of tryptase-positive cells per mm².

Expression of results and statistical analysis

Results of SCF protein and mast cell numbers are expressed as means ± SEM. Results of SCF mRNA are expressed as geometrical means ± SEM because of a variance-stabilizing log transformation. Statistical analysis of the results used an unpaired *t*-test. A *P* < 0.05 was considered significant.

Results

Stem cell factor protein levels in bronchoalveolar lavage fluid

SCF protein was detectable in the BALF samples from all patients. Volunteers from the control group exhibited SCF protein levels with a mean value of 56.0 ± 11.0 pg/mL (Fig. 1). Asthmatic patients not treated by glucocorticoids showed a 1.8-fold significant increase in SCF protein levels as compared with volunteers of the normal control group (99.8 ± 11.5 pg/mL; *P* < 0.05) (Fig. 1). Asthmatic patients treated with glucocorticoids showed a significant decrease in the SCF protein levels as compared with untreated asthmatic patients (62.0 ± 10.3 pg/mL; *P* < 0.05), reaching SCF protein levels comparable to those of the control group (Fig. 1).

Stem cell factor messenger RNA expression in bronchial biopsies

SCF mRNA was detectable in every sample. Volunteers from the control group exhibited SCF mRNA levels with a

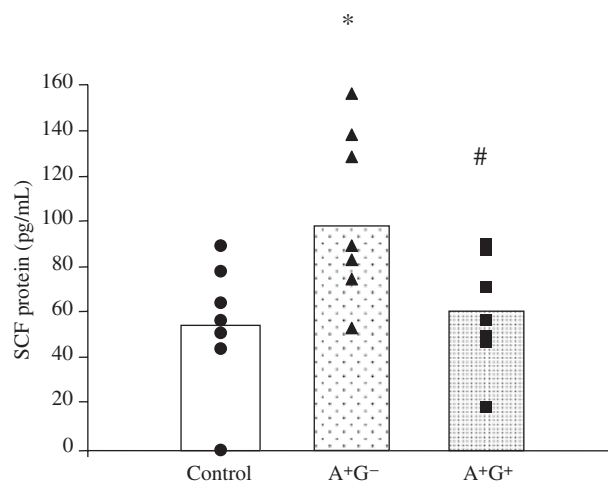


Fig. 1. Stem cell factor (SCF) protein levels in bronchoalveolar lavage fluid (BALF). SCF protein levels were assessed by enzyme-linked immunosorbent assay in BALF from control subjects (control: open block), asthmatic patients not treated (A⁺G⁻: dotted blocks) and treated with glucocorticoids (A⁺G⁺: strong dotted blocks). Results are expressed as pg/mL, and are represented as individual (dots) and mean values (blocks). **P* < 0.05 as compared with control, and # *P* < 0.05 as compared with A⁺G⁻.

geometric mean value of 1.3 pg/ μ g (Fig. 2). Asthmatic patients untreated by glucocorticoids showed a 5.8-fold significant increase in SCF mRNA expression as compared with healthy controls (7.8 pg/ μ g; $P < 0.05$) (Fig. 2). Asthmatic patients treated with glucocorticoids showed a significant decrease of the SCF mRNA levels compared with untreated asthmatic patients (1.1 pg/ μ g; $P < 0.05$), reaching SCF mRNA levels comparable to those of the control group (Fig. 2).

Mast cell numbers in bronchial biopsies

Volunteers from the control group exhibited low numbers of mast cells infiltrated within the airways, with a mean value of 33 ± 8 mast cells/mm² (Fig. 3). Mast cells were localized within the bronchial epithelium (2 ± 1 /mm²), the submucosa (29 ± 8 /mm²) and the bronchial smooth muscle (2 ± 1 /mm²). They were mainly not degranulated. Asthmatic patients untreated with glucocorticoids showed a 3.1-fold significant increase in mast cell numbers in the bronchial biopsies compared with volunteers of the control group (103 ± 21 mast cells/mm²; $P < 0.05$) (Fig. 3). Mast cells increased by 7.5-fold within the bronchial epithelium (15 ± 4 /mm²), 2.5-fold in the submucosa (73 ± 15 /mm²) and 6-fold within the bronchial smooth muscle (12 ± 5 /mm²). They were very often degranulated. Asthmatic patients treated with glucocorticoids showed a significant decrease in mast cell numbers in the bronchial biopsies compared with untreated asthmatic patients

(39 ± 13 mast cells/mm²; $P < 0.05$), reaching mast cell numbers comparable to those of the control group (Fig. 3). They were mainly localized to the bronchial epithelium (5 ± 3 /mm², 3-fold decrease), submucosa (30 ± 11 /mm², 2.4-fold decrease) and bronchial smooth muscle (4 ± 2 /mm², 3-fold decrease).

Discussion

The present study provides evidence of the mast cell growth factor SCF production in the human bronchus *in vivo*. The expression of both SCF protein and mRNA levels is elevated locally in the airways of asthmatic patients as compared with healthy subjects, which is associated with elevated mast cell numbers. More importantly, our study reports decreased SCF mRNA and protein levels and mast cell numbers within the airways in glucocorticoid-treated asthma patients.

First, our data show that SCF protein and mRNA can be detected in BALF and bronchial biopsy specimens, respectively, both from human control and asthmatic subjects. This may be related to the constitutive and stimulated expression of SCF reported *in vivo* [18, 20], or *in vitro* by airway structural cells in culture, like lung fibroblasts [24, 32, 33] or airway smooth muscle cells [25].

Additionally, here, we show that SCF expression is increased in the airways in asthma as compared with airways from control non-asthmatic subjects. This finding is in agreement with the increased SCF mRNA and protein expression recently reported in asthmatic patients [18]. This is furthermore in agreement with the overexpressed

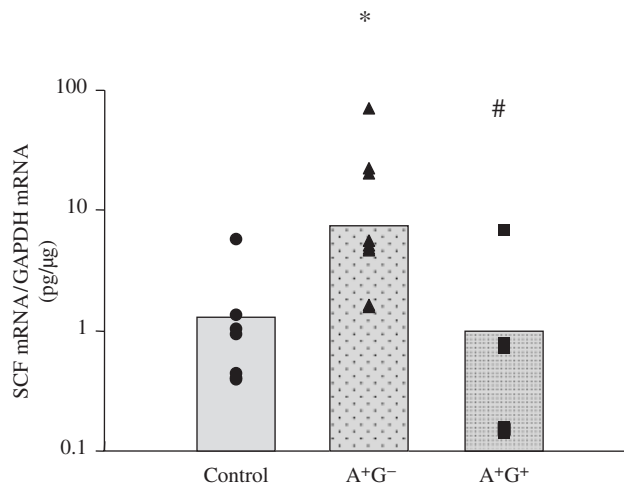


Fig. 2. Stem cell factor (SCF) messenger RNA (mRNA) in bronchial biopsies. SCF mRNA was quantified after total RNA reverse transcription by on-line fluorescent PCR in bronchial biopsy specimens from control subjects (control: pale grey, open block), asthmatic patients not treated (A⁺G⁻: pale grey dotted blocks) and treated with glucocorticoids (A⁺G⁺: pale grey, strong dotted blocks). Results were normalized to GAPDH mRNA expression, and expressed in pg/ μ g as the ratio (SCF mRNA)/(GAPDH mRNA). Results are represented as individual (dots) and geometric mean values (blocks). * $P < 0.05$ as compared with control, and # $P < 0.05$ as compared with A⁺G⁻.

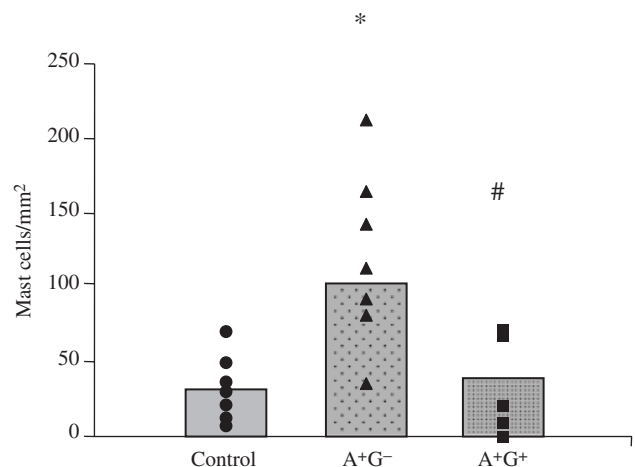


Fig. 3. Mast cell counts. Mast cell counts were performed using a mouse monoclonal antibody raised against human tryptase and revealed by FastBlue[®] in bronchial biopsy specimens from control subjects (control: dark grey open block), asthmatic patients not treated (A⁺G⁻: dark grey dotted blocks) and treated with glucocorticoids (A⁺G⁺: dark grey strong dotted blocks). Results are expressed as mast cells/mm². Results are individual (dots) and mean values (blocks). * $P < 0.05$ as compared with control, and # $P < 0.05$ as compared with A⁺G⁻.

levels of SCF mRNA and protein in the asthmatic airways in a murine model of allergic bronchial inflammation, where exposure to allergen led to increased SCF protein levels in the lung and in serum [19]. In these experiments, intra-tracheal administration of an antibody directed against SCF protein led to a decrease in the allergen-induced inflammation in the lung [19], suggesting a role for SCF in the inflammatory process of asthma. This increase may be due to SCF expressed by inflammatory cells whose number is increased in the airways, like the mast cells themselves reported to express SCF [26, 27], as well as by structural cells. Indeed, we previously reported *in vitro* that SCF mRNA and protein expression is increased after treatment with IL-1 β , a major pro-inflammatory mediator present at high levels in the asthmatic airways [34], in human lung fibroblasts [24, 32] or human airway smooth muscle cells in culture [25]. This is in accordance with a report of an increased number of nasal ciliated epithelial cells expressing SCF protein and mRNA in the upper airways of patients with seasonal allergic rhinitis, another mast cell-associated disease, as compared with control subjects [20]. In addition, SCF expression was recently mainly localized to the epithelium layer in bronchial biopsy sections [18]. We may thus submit that the structural cells of the airways may participate in the increased expression of SCF in asthma. As asthma is a mast cell-dependent disease and SCF is a major mast cell growth factor, promoting chemotaxis and activation of mast cells [7, 11], our data and those of others allow to propose SCF as being involved in the inflammatory process of the increased number of mast cells in asthma in humans.

Indeed, our study confirms that mast cell numbers are increased within the airways of asthmatic patients, being mainly located within the bronchial epithelium, the airway smooth muscle and the submucosa. These findings are in agreement with reports of increased mast cell numbers within the airways of asthmatic patients even in the absence of allergen exposure [13–15, 35–37], and with the reported location of mast cells within the bronchial epithelium [13, 15]. These findings also confirm very recent studies performed by Amin et al. [35], showing a significant increase in mast cell numbers within the bronchial smooth muscle layer. Finally, our results are also in accordance with previous findings that the infiltrated mast cells are mainly degranulated [38].

More importantly, our study shows that patients treated with glucocorticoids exhibited decreased levels of SCF protein in BALF and SCF mRNA in bronchial biopsy specimens as compared with untreated asthmatics, thus returning to levels comparable to those of control patients. It should be noted that the glucocorticoid-treated patients recruited were significantly older than the healthy controls, and exhibited more severe asthma, which was controlled by glucocorticoids as indicated by

the PD₂₀ values. Therefore, this cross-sectional study indicating control of airway hyper-responsiveness by glucocorticoids was also associated with a decreased expression of SCF in the airways. This finding is in accordance with a decreased number of structural cells expressing SCF in patients with nasal polyposis treated with glucocorticoids [20]. It is also in agreement with a decrease of the SCF immunoreactivity of structural cells reported in mouse dermis after treatment with glucocorticoids [39]. In addition, we previously reported that the glucocorticoid budesonide decreases SCF mRNA and protein expression induced by IL-1 β *in vitro* in human lung fibroblasts in culture [24], which is also in agreement with the results presented here of a decreased SCF expression in the anti-inflammatory conditions of the asthmatic airways during treatment with glucocorticoids *in vivo*. These results altogether show that glucocorticoids, even in mild to moderate asthma, can decrease SCF expression in human airways to levels comparable to those of healthy subjects. The decreased SCF expression may alternatively originate from the decreased inflammatory cell infiltrate expressing SCF in the airways. Glucocorticoids are the major treatment for asthmatic disease, and are reported to reduce mast cell numbers, in particular, in the bronchial mucosa of asthmatic patients [40–42]. Here, we confirm that treatment with glucocorticoids was associated with a decrease in mast cell number within the airways of asthmatic patients, returning to levels similar to those found in control subjects. This is also in agreement with immuno-histochemical studies *in vitro*, showing reduction by glucocorticoids of the cutaneous mast cell numbers by increasing their apoptosis [39]. All these findings allow to submit that inhibition of SCF expression by glucocorticoid treatment may have a role, at least in part, in the reduced mast cell number and activation in the airways, and in a subsequent decrease in the inflammatory processes observed in the asthmatic disease after treatment with glucocorticoids [40].

Recent studies showed that SCF could also act together with other growth factors, like transforming growth factor (TGF)- β [43, 44], IL-9 [45, 46] or nerve growth factor (NGF) [47, 48], to increase mast cell numbers within the asthmatic airways. Since decreased expression of these growth factors has also been reported by glucocorticoids [49–51], these data allow, altogether, to propose SCF, alone or in combination with other mediators, as being involved in the inflammatory process of the increased number of mast cells in asthma in humans.

In conclusion, our study clearly shows that SCF mRNA and protein are expressed in the bronchus in humans *in vivo*, and that this expression is increased in the inflammatory conditions of the asthmatic bronchus in patients not treated by glucocorticoids, in parallel to the increase in mast cell infiltration in the airways. Additionally, asthmatic patients treated with glucocorticoids exhibit

normalized levels of SCF protein and mRNA in the bronchus, as well as of mast cell numbers, all of them returning to levels comparable to those of the airways of control subjects. Therefore, as expected from *in vitro* studies performed on airway cells in culture [24, 32], we may propose SCF as a pro-inflammatory mediator implicated in the mast-cell-associated asthmatic condition.

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