

ORIGINAL PAPER

Influence of smoking on airway inflammation and remodelling in asthma

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Clinical and
Experimental
Allergy

Summary

Background Although exposure to tobacco smoke has been associated with increased morbidity and mortality, cigarette smoking is still common in the asthmatic population. Induced sputum neutrophilia has been observed in asthmatic smokers, but the effects of regular smoking on their bronchial mucosa morphology remain to be defined. This study documents the inflammatory and remodelling features in bronchial biopsies of smoking compared with non-smoking asthmatics.

Methods We analysed bronchial biopsies from 24 steroid-naïve young subjects with mild asthma: 12 non-smoking and 12 currently smoking subjects. In addition to airway morphology assessment, inflammation and remodelling were analysed by immunohistochemistry using antibodies against CD3, CD68, major basic protein, neutrophil elastase, and tryptase. Expression of the cytokines IL-4, IL-5, IL-8, IFN- γ , transforming growth factor- β , and TNF was determined by *in situ* hybridization.

Results Compared with non-smoking asthmatic subjects, smoking asthmatics' bronchial mucosa showed squamous cell metaplasia, in addition to increased expression of subepithelial neutrophil elastase, IFN- γ , and intraepithelial IL-8.

Conclusions Smoking status modifies morphological and inflammatory processes in young subjects with mild asthma. The changes may possibly affect asthma treatment responses and clinical outcomes.

Keywords airway inflammation, asthma, bronchial biopsies, cigarette smoking

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Introduction

Smoking is common in the general population and cigarette smoke is implicated in airway damage [1]. Lung cancer, chronic pulmonary disease, and asthma are diseases linked to the consumption of tobacco. Indeed, in non-asthmatic smokers, a positive correlation has been observed between major basic protein (MBP)-positive cells in the subepithelial layer of bronchial biopsies and forced expiratory volume in 1 s (FEV₁) [2]. Moreover, an increase in inflammatory cells such as neutrophils, eosinophils, mast cells, and macrophages, and in cytokines such as IL-1 β and IL-8 has also been reported in bronchial biopsies from non-asthmatic smokers compared with non-smokers [3, 4]. Finally, structural changes have been documented in the bronchial mucosa of non-asthmatic smokers, such as an increased deposition of tenascin and laminin under the basement membrane [3].

A large proportion of smokers have asthma, the prevalence of smoking in the asthmatic population being between 25% and 30% in Canada [1, 5]. Asthma is characterized by inflammatory cell infiltration of the mucosa and a remodelling process that includes epithelial damage, glandular hyperplasia, subepithelial fibrosis, smooth muscle hyperplasia/hypertrophy, and angiogenesis [6, 7]. These changes have been implicated in the decline in pulmonary function and in the persistence of airway hyperresponsiveness in asthmatic patients [7–9]. Environmental exposure to allergens or sensitizing industrial agents may promote airway inflammation and remodelling in susceptible individuals. However, the effects of substances such as pollutants and tobacco smoke remain unclear [10, 11].

Epidemiological evidences suggest that smoking has a marked detrimental effect on lung function in asthmatic subjects [8]. Smoking has been associated with increased

asthma-related morbidity, healthcare use, accelerated decline in pulmonary function, and an increased risk of severe asthma exacerbation [8, 12–16]. Moreover, it has been reported that cigarette smoke influences the efficiency of short-term inhaled corticosteroid treatment in mild asthmatic subjects [17].

Little is known on the effects of smoking as a contributing factor or modifier of inflammatory and structural changes of airway mucosa in asthma. It has been shown that cigarette smoke modifies the airway inflammatory profile, neutrophils being observed in increased numbers in induced sputum of asthmatic smokers compared with non-smokers [17, 18]. Furthermore, cigarette smoke alters inflammatory cell populations both in peripheral blood and in airway fluids/mucosa independently of airway obstruction [19, 20]. Recently, smoking has been associated with a reduction in the numbers of CD83 mature dendritic cells and B cell numbers [21]. Bergeron et al. [22] recently showed an increased expression of arginase I and ornithine decarboxylase in airways of smoking compared with non-smoking asthmatic subjects. *In vitro* exposition of nicotine of BEAS-2B-derived airway epithelial cell line, smooth muscle cells, and fibroblasts resulted in an increased expression of arginase I, which might be responsible for the low exhaled nitric oxide and chronic obstructive pulmonary disease (COPD)-like airway changes observed in smoking asthmatic patients.

There is a need to further document the effects of smoke on airway morphological and inflammatory changes in asthmatic subjects. We hypothesized that cigarette smoke induces airway mucosa changes in young asthmatic smokers, similar to early-COPD changes. The present study compares bronchial biopsies from smoking and non-smoking young subjects with mild asthma to document the morphological inflammatory and remodelling features of mucosa.

Material and methods

Patient population and study design

Biopsies from 24 steroid-naïve mild asthmatic subjects meeting the criteria of the American Thoracic Society (ATS) were analysed [23]. Twelve smoking asthmatic subjects were recruited from the Laval Hospital Asthma Clinic (Québec, Canada) and tissues from 12 non-smoking asthmatics meeting the same criteria were obtained from the Quebec Respiratory Health Network Tissue Bank at Laval Hospital. The non-smoking group included never-smokers and subjects who had not smoked in the last year and who smoked less than two pack-years. All subjects were in good health apart from having asthma, used only inhaled short-acting β_2 agonists when necessary to treat their asthma symptoms, and had no history of respiratory infection or asthma exacerbation in the last 6 weeks. Expiratory flows were measured according to the ATS

criteria on an ATS-approved spirometer [24]. Methacholine responsiveness was measured using the tidal volume method described by Juniper [25]. All subjects provided an informed consent and the study was approved by the Laval Hospital Ethics Committee.

Bronchoscopy and tissue processing

Bronchial biopsies were obtained from all patients according to current guidelines [26]. Bronchoscopy was performed by only one endoscopist (M. L.) and biopsies were taken at the bronchial sub-segmental or lobar divisions. The Tissue Bank at Laval Hospital processed all biopsies from non-smoker and smoker asthmatics using the same standardized methodology. Tissues were fixed in 4% paraformaldehyde, embedded in paraffin [for histology and immunohistochemistry (IHC)], and kept at room temperature, or blocked in optimal cutting temperature (OCT) embedding medium (Sakura Finetek Inc., Torrance, CA, USA), snap-frozen in liquid nitrogen-cooled isopentane [for *in situ* hybridization (ISH)], and kept frozen at -80°C until use. For both groups, the same method for conservation of tissues was used. Sections ($5\mu\text{m}$) were cut using a microtome (Surgipath Medical Industries, Richmond, IL, USA) or a cryostat (Microm International, Walldorf, Germany).

Morphological analyses of bronchial biopsies

Paraffin sections were stained using Masson's Trichrome method according to the manufacturer's instructions (Sigma Diagnostics, St. Louis, MO, USA) for histological and morphological evaluation.

Complete evaluation of bronchial epithelium, including epithelial integrity, goblet cell hyperplasia, squamous cell metaplasia, and basement membrane thickness was performed. A quantitative evaluation of epithelium morphology was performed. Bronchial epithelium was considered intact (ciliated pseudostratified epithelial cells including basal and columnar cells), partially shedded (loss of cilia, absence of stratification, residual basal cells), or completely shedded (total shedding of the epithelium), and evaluated as percentage of total length of the basement membrane. A qualitative assessment of the presence of goblet cells within the bronchial epithelium and squamous cell metaplasia was carried out. Squamous cell metaplasia has been characterized as stratified epithelium, with flattened epithelial cells on the surface.

Analyses of the bronchial mucosa including smooth muscle area, submucosal gland area, and reticular basement membrane (RBM) thickening associated with subepithelial fibrosis measurements were performed. Morphological parameters were quantified using digital images obtained with an Eclipse 600 light microscope (Nikon Canada Inc., Mississauga, ON, Canada) equipped with a digital camera (SPOT RT rider, Diagnostic Instruments, Sterling Heights,

MI, USA), with the SimplePCI Image analysis software (Compix Inc. Imaging Systems, Cranberry Township, PA, USA). To compare morphological characteristics between different biopsies, ratios were established as the percentage of submucosal gland, smooth muscle, or connective tissue areas on the total bronchial biopsy area, excluding bronchial epithelium. The thickness of RBM was measured at three different locations along the RBM associated with an intact epithelium. For each subject, the mean value of these three measurements was calculated. Evaluation was performed by two blinded observers. The between-observers results showed no significant difference ($P > 0.05$).

Inflammatory cell counts and remodelling

Subepithelial inflammation was evaluated by IHC. First, slides were deparaffinized in xylene, followed by decreasing concentrations of ethanol. Immunostaining of biopsy sections was assessed using the Dako EnVision+HRP system (Dako, Mississauga, ON, Canada). Briefly, following the specific antigen retrieval method (10 mM sodium citrate, pH 6.0; 0.1% trypsin; 0.1% pepsin; 10 mM Tris, 1 mM EDTA, pH 9.0; or none), sample peroxidase activity and non-specific background were blocked by successive incubation with 3% hydrogen peroxide (H_2O_2) in Tris buffer solution (TBS) for 30 min and Protein Block Serum-Free (Dako) for 30 min. Mouse monoclonal anti-human CD3 (15 μ g/mL), CD68 (10 μ g/mL), neutrophil elastase (10 μ g/mL), tryptase (1 μ g/mL; Dako), MBP (20 μ g/mL; Biotest International, Saco, MA, USA), decorin (10 μ g/mL; R&D Systems Inc., Minneapolis, MN, USA), versican (0.5 μ g/mL; US Biologicals, Massachusetts, MA, USA), rabbit polyclonal anti-human collagen I (1 : 550) and III (1 : 200; Cedarlane Laboratories Ltd., Hornby, ON, Canada) or fibronectin (3 μ g/mL; Biomed, Foster City, CA, USA) primary antibodies were then added and incubated at 4 °C overnight. Mouse-HRP-labelled EnVision+ reagent was added and detection was performed using AEC+ substrate-chromogen. Slides were counterstained with Mayer's haematoxylin (Dako) and mounted with Crystal Mount (Biomed). Washes with TBS were performed between steps. Irrelevant isotypic antibodies (mouse IgG, 10 μ g/mL; Dako) were used as negative controls. Inflammatory cells were expressed as the number of positive cells in the non-muscular, non-cartilaginous, non-glandular connective tissue per square millimetre (mm^2) of submucosal area, excluding the epithelium. The slides were analysed blindly by two different observers and their results were similar ($P > 0.05$).

Probe preparation and in situ hybridization

Ribonucleic acid (RNA) probes coding for IL-4, IL-5, IL-8, IFN- γ , TNF and transforming growth factor (TGF)- β mRNA were prepared from complementary deoxyribonu-

cleic acid (cDNA) as described previously [27, 28]. Briefly, cDNA was inserted into pGEM vectors, linearized, and transcribed *in vitro* in the presence of α - ^{35} S-UTP and either SP6 or T7 polymerases. Antisense (complementary to mRNA) and sense probes (identical to mRNA, used as negative controls) were prepared. Sections of bronchial biopsy tissues were processed for ISH with radiolabelled [α - ^{35} S-UTP] sense and antisense probes, hybridized, and washed under high-stringency conditions, as described previously [27, 28]. All slides were observed under dark-field microscopy and positive signals appeared as bright light. Positive cells were counted blindly by two different experienced observers and expressed per mm^2 of submucosa. Areas of the biopsies were measured under light microscopy. A scoring technique from 0 to 8, widely used in our laboratory, was applied to quantify the positivity in the epithelium for IL-8 detected by ISH (0 = negative staining, 1 = 1–12.5%, 2 = 12.5–25%, etc. . . , 8 = 87.5–100%).

Statistical analysis

Mean and standard error of the mean were used for continuous variables. Comparisons between non-smoking and smoking asthmatic subjects were made using a one-way ANOVA (clinical and morphological characteristics and *in situ* hybridization) or the Wilcoxon rank sum test (inflammatory cell counts). Normality and variance assumptions based on tests and graphical representations were used to validate statistical analyses. All reported *P*-values are two-sided. Semiquantitative data were analysed using χ^2 contingency tables. All results were considered to be significant with *P*-values ≤ 0.05 . These data were analysed using the statistical package program SAS v9.1.3 (SAS Institute Inc, Cary, NC, USA).

Results

Clinical characteristics

The clinical characteristics of subjects (Table 1) were similar in smoking and non-smoking asthmatic subjects, except for a higher forced vital capacity (FVC) in asthmatic smokers. The FEV₁, the ratio FEV₁/FVC, and the forced expiratory flow (FEF_{25–75%}) were not different between smokers and non-smokers. Subjects in the smokers group had smoked from 6 to 30 pack-years, these subjects being young adult smokers with a moderate smoking habit.

Bronchial subepithelial mucosa morphology

The smooth muscle bundles, subepithelial gland, and connective tissue areas were observed in the same ratio of the total biopsy for both groups, ($P > 0.05$). Moreover,

Table 1. Subjects' characteristics

	Non-smoking asthmatics (n = 12)	Smoking asthmatics (n = 12)
Age (years)	25.8 ± 2.3	32.7 ± 2.3
Gender (M/F)	3/9	4/8
Atopy (yes/no)	10/2	7/5
FEV ₁ (% pred)	92.3 ± 4.7	88.4 ± 4.4
FVC (% pred) [†]	97.4 ± 5.3	108.6 ± 4.6
FEV ₁ /FVC (%)	79.2 ± 2.2	69.7 ± 2.4
FEF _{25–75%} (% pred)	71.8 ± 6.6	62.6 ± 6.0
PC ₂₀ (mg/mL)*	1.5 ± 1.3 (0.3–15.4)	1.7 ± 0.9 (0.5–8.6)
Duration of asthma (years)	15.3 ± 2.5	15.7 ± 3.1
Smoking history (pack-year)	none	16.7 ± 2.2 (6–30)
Current smoking (cigarettes/ day)	none	19.5 ± 3.0 (12–50)

Results are expressed as mean ± SEM (range).

*Geometric mean ± SEM (range).

[†]P-value < 0.05 between both group.

FVC, forced vital capacity; FEV, forced expiratory volume in 1 s; FEF, forced expiratory flow.

Table 2. Morphological changes in bronchial epithelium and submucosa

	Non-smoking asthmatics	Smoking asthmatics
Smooth muscle area/total biopsy area (%)	13 (4; 19)	12 (8; 21)
Submucosal gland area/total biopsy area (%)	0 (0; 8)	3 (0; 5)
Connective tissue area/total biopsy area (%)	83 (72; 87)	81 (68; 88)
Basement membrane thickness (µm)	13 (12; 16)	13 (11; 16)
Squamous cell metaplasia (yes/no)*,†	0/12	3/9
Goblet cell hyperplasia (yes/no)*	2/10	5/7
Intact epithelium/total length of basement membrane (%)	9 (0; 17)	14 (9; 21)
Partially shedded epithelium/total length of basement membrane (%)	69 (50; 78)	53 (33; 71)
Completely shedded epithelium/total length of basement membrane (%)	25 (16; 31)	18 (10; 29)

Results are expressed as median (25; 75th percentiles).

*Qualitative analysis see morphological analyses of bronchial biopsies in methods section.

[†]P-value = 0.08.

the thickness of the RBM was identical in both groups, $P > 0.05$ (Table 2).

Bronchial epithelial cell morphology

The percentage of intact, partially, or completely shedded epithelium was similar for both groups ($P > 0.05$). Goblet cell hyperplasia was observed in five out of 12 smoking asthmatics and in only two of the 12 non-smoking

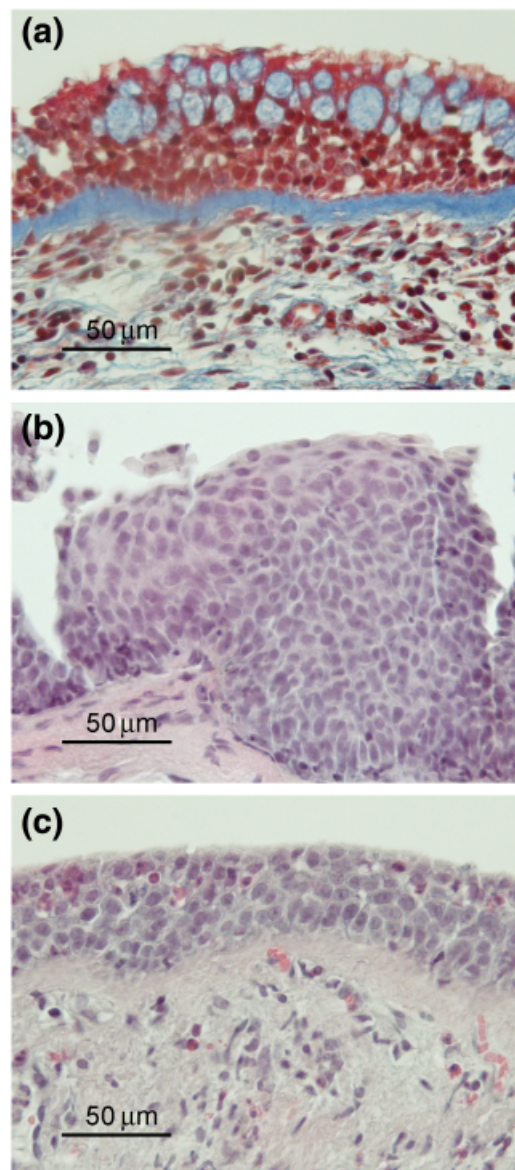


Fig. 1. Goblet cell hyperplasia with reticular basement membrane fibrotic thickening (a), squamous cell metaplasia (b), and neutrophils infiltration (c) observed in bronchial biopsies from a smoking asthmatic subject.

asthmatics (Fig. 1a and Table 2). Squamous cell metaplasia was noted only in biopsies from smoking asthmatics (three out of 12 smoking patients) (Fig. 1b and Table 2).

Airway mucosa cell infiltrate

The numbers of neutrophil elastase-positive cells were increased in smoking compared to non-smoking asthmatics (Fig. 1c): 533 (248; 1044) and 32 (0; 128) cells/mm² ($P < 0.05$), respectively. CD3+, CD68+, MBP+, and tryptase+ cells were not different between groups ($P > 0.05$) (Table 3). Tryptase-positive cells were found

Table 3. Bronchial inflammatory features

	Non-smoking asthmatics	Smoking asthmatics
CD3+ cells	50 (0; 233)	502 (84; 1209)
CD68+ cells	3430 (2062; 4466)	2785 (1699; 5939)
MBP+ cells	0 (0; 165)	35 (0; 109)
Elastase+ cells	32 (0; 128)	533 (248; 1044) [†]
Tryptase+ cells	8474 (5382; 12 164)	8721 (7899; 9759)
IL-4	13.6 (7.1; 49.2)	15.4 (8.6; 34.6)
IL-5	26.5 (14.6; 48.4)	32.4 (7.0; 39.2)
IL-8 (submucosa)	26.3 (10.2; 45.1)	34.6 (22.6; 46.2)
IL-8 (intraepithelial)*	0.6 (0.5; 0.94)	2.0 (1.25; 2.5) [†]
IFN- γ	8.4 (5.6; 11.0)	19.7 (15.4; 31.4) [†]
TNF	22.0 (9.6; 46.4)	23.8 (16.3; 34.5)
TGF- β	19.5 (17.8; 25.4)	25.5 (13.1; 35.7)

Positively stained cells/mm² of connective tissue expressed as median (25; 75th percentiles)

*Qualitative scoring technique (0–8) see probe preparation and ISH in methods section

[†]*P*-value < 0.05.

TGF, transforming growth factor; ISH, *in situ* hybridization; MBP, major basic protein.

around the smooth muscle bundles, subepithelial glands, and in connective tissue.

IFN- γ expression in the bronchial mucosa of smoking asthmatics was higher than that in non-smoking asthmatics: 19.7 (15.4; 31.4) and 8.4 (5.6; 11.0) cells/mm², respectively (*P* < 0.05) (Table 3). IL-4, IL-5, IL-8, TNF, and TGF- β expressions were equivalent between groups in the submucosal cells. As the main source of IL-8 is epithelial cells, it was not surprising to observe a positive signal in the epithelium. An increased expression of intraepithelial IL-8 in smoking asthmatics compared with non-smoking asthmatics was observed [epithelial score: 2.0 (1.25; 2.5) vs. 0.6 (0.5; 0.94), respectively, *P* < 0.05] (Table 3).

ECM components

Immunohistochemical staining was performed for collagens I and III, fibronectin, and proteoglycans (versican and decorin) to establish the distribution pattern and intensity of ECM proteins. Qualitative analyses were carried out. Decorin and versican were only expressed in the connective tissue; epithelial and endothelial cells, smooth muscle bundles, RBM, and mucous and serous cells of submucosal glands were consistently negative. In some subjects, versican staining was more intense around smooth muscle bundles, but no difference was noted between non-smoking and smoking asthmatic subjects. Type I and III collagens and fibronectin showed similar staining in the connective tissue, with additional staining beneath the basement membrane explaining the apparent RBM thickness associated with asthma. No significant differences were noticed between the two groups (data not shown).

Discussion

This study evaluated the effects of cigarette smoke on histopathological features of the asthmatic bronchial mucosa. It showed that smoking modifies asthmatic airways, inducing squamous cell metaplasia, increasing neutrophil infiltration and expression of IFN- γ in bronchial biopsies. Expression of intraepithelial IL-8 mRNA was also increased in asthmatic smokers compared with non-smokers. For lymphocytes and eosinophils, there was a large inter-subject variability that may explain the absence of significant differences between the two groups.

Our study included young asthmatics with moderate smoking habits. They demonstrated only a few significant differences compared with non-smokers due to their young age, their mild asthma intensity, and their mild smoking exposure. These differences may represent the initiation of smoking damage. Other studies on asthmatic and non-asthmatic subjects have demonstrated more marked differences between smoker and non-smoker in older subjects [3, 16, 29].

A comparison between non-smoking and smoking healthy subjects would have been interesting, but this has been published previously [3, 4, 19] and the aim of this study was mainly to look at how smoking may influence asthma inflammation and remodelling features. Previous reports had shown that cigarette smoke induced inflammatory changes in the airway wall of non-asthmatic subjects with increases in neutrophils, eosinophils, mast cells, and macrophages, in addition to remodelling changes such as tenascin and laminin deposition under the basement membrane [3, 4, 19].

In our study, airway epithelium in smoking asthmatics showed squamous cell metaplasia. It is known that chronic inflammatory injury, such as cigarette smoke, causes damage of airway epithelium and initiates an airway inflammatory response [30, 31]. After repeated damage in the epithelium, epithelial cells migrate to the denuded area, start proliferating and establish squamous or mucous cell metaplasia [32]. Arginase I is implicated in cell proliferation and mucus production, and can stimulate the proliferation of squamous cell metaplasia and goblet cell hyperplasia. Bergeron et al. [22] showed that arginase I was increased in asthmatic smokers' mucosa. Moreover, in non-asthmatic smoking subjects and COPD patients, squamous cell metaplasia and goblet cell hyperplasia are also observed [3, 33].

Cigarette smoke is a common and important aggression to the airways that stimulates inflammatory cells to produce cytokines [34]. In our study, expression of IFN- γ in the mucosa was increased in asthmatic smokers compared with asthmatic non-smokers. IFN- γ has diverse immunoregulatory effects on various cells and may induce airway epithelial cells' release of cytokines [35]. A higher expression of IFN- γ might not only lead to

structural alteration but might also influence the response to corticosteroids. Recently, a high level of expression of IFN- γ has been associated with relative corticosteroid insensitivity in peripheral blood mononuclear cells (PBMC) of severe asthma through a reduction in histone deacetylase activity [36].

In our study, expression of IL-8 was high in the epithelium of the smoking group, which was not surprising knowing that IL-8 is mainly produced by epithelial cells [37]. An increased production of IL-8 is observed in the sputum from smoking asthmatic subjects. Increased airway IL-8 is also observed in severe asthma and COPD patients [18, 38, 39]. IL-8 is well known to promote the recruitment of neutrophils in the airway lumen [18, 39–41]. Consequently, the increased intra-epithelial IL-8 expression is likely involved in the recruitment of neutrophils in airway mucosa.

In the present study, the airway wall of asthmatic smokers showed a higher number of neutrophil elastase-positive cells than in asthmatic non-smokers. Consequently, our study demonstrated that smoking increased neutrophil number not only in the airway lumen, as reported previously, but also in airway mucosa of subjects with mild asthma, which we believe is the result of increased IL-8 production by epithelial cells. In mice, exposure to tobacco induces chronic lung inflammation with increased numbers of neutrophils, macrophages, and lymphocytes associated with a decreased lung elasticity and tissue destruction [42, 43]. Neutrophil infiltration is known to increase the production of mucus, an important effect of cigarette smoke exposure [44, 45]. Also, neutrophils are believed to be corticosteroid resistant through a decreased apoptosis rate and a higher expression of the dominant-negative GR β isoform [46]. Together, increased IFN- γ , IL-8 expression, and neutrophil infiltration may alter the physiological functions of airway structural cells (including epithelial cells), could increase mucus production, and contribute to airflow obstruction [45] and corticosteroid resistance [36].

Moreover, cigarette smoking is the most important risk factor for the development of COPD [47]. Epithelial structural changes have been observed after chemical damage in airways of smoking asthmatic subjects and these modifications of bronchial epithelium are well documented in COPD [33]. Airway inflammation in COPD is characterized by an increased number of macrophages and neutrophils in sputum and bronchoalveolar lavages, and of T lymphocytes and macrophages in bronchial biopsies [48]. At an early stage of COPD, neutrophil intraepithelial and lymphocyte mucosal infiltrations, in addition to IFN- γ and IL-8 expression, are considered to play a key role in the progression of the disease [40, 45, 49]. Boulet et al. [18] observed that young smoking asthmatic subjects had, in addition to an asthma phenotype, a lower mid-expiratory flow, FEV₁/FVC ratio, and

lung diffusion capacity, a higher functional residual capacity, and an increased number of neutrophils in the sputum. Moreover, airway and parenchymal abnormalities were more often observed on chest tomodensitometry in smoking than in non-smoking asthmatic subjects. These observations, in addition to our results, support the hypothesis that young asthmatic smokers develop early COPD-like alterations.

One of the limitations of this study was the number of subjects in each study group involving bronchial biopsies. Twenty-four subjects were recruited and risk of a type-2 error was possible. However, some features were significantly different between the two groups.

In conclusion, this study suggests that cigarette smoking in young asthmatic subjects could contribute to early COPD-like airway changes that may possibly modify clinical outcomes and asthma treatment response. Further studies should be performed to determine the time-course of the observed changes and the influence of smoking cessation on asthma and response to therapy. The mechanisms by which smoking could influence the basic physiopathological process of asthma should still be explored, as should the influence of smoking cessation on the reversibility of these features. Needless to say, smoking cessation should be promoted in all asthmatic subjects, in order to improve control of the disease and prevent further decline in pulmonary function and airway damage.

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