

# Isolated airways from current smokers are hyper-responsive to histamine

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## Summary

**Background** Epidemiological studies suggest that bronchial hyper-responsiveness (BHR) and elevated levels of serum IgE are more frequently found in current smokers than in ex-smokers.

**Objective** Since elevated serum IgE is associated with BHR under both *in vivo* and *in vitro* conditions, we aimed to assess whether smoking affects BHR independently from IgE.

**Methods** Lung resection material was obtained from 27 current smokers and 11 non-smokers with low serum IgE (< 100 U/mL). Peripheral airways were cut into rings and incubated overnight in the presence (passively sensitized) or absence (non-sensitized) of serum containing IgE levels above 250 U/mL. Isometric contractile responses to histamine were assessed in the organ bath.

**Results** Compared with non-smokers, isolated airways from smokers showed significantly increased responses to histamine ( $P < 0.05$ , ANOVA). Passive sensitization enhanced responses in both groups by about the same amount ( $P < 0.05$ , both).

**Conclusions** In patients with low serum IgE current smoking is associated with increased bronchial responsiveness to histamine *in vitro*, which can be further enhanced by passive sensitization. These findings suggest that both smoking and serum IgE contribute to non-specific airway hyper-responsiveness.

**Keywords:** smoking, IgE, airway smooth muscle, bronchial hyper-responsiveness, passive sensitization, asthma, COPD, human, *in vivo*, *in vitro*

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## Introduction

Chronic airway diseases such as bronchial asthma and chronic bronchitis are characterized by airway hyper-responsiveness to non-specific stimuli [1–4]. Although different alterations within the airways appear to be involved, the mechanisms underlying the bronchial hyper-responsiveness in both diseases are not fully understood. They are believed to be primarily the consequence of inflammation and changes in smooth muscle physiology [5] and/or airway structure [6]. Epidemiological studies indicate that elevated levels of serum IgE [7,8], as well

as active smoking [9], are associated with bronchial hyper-responsiveness. Although both factors appear to be related to each other [10], independent effects of smoking and IgE on airway function have also been suggested [11].

In a previous study we found that total serum IgE was an *in vivo* determinant of airway smooth muscle hyper-reactivity *in vitro* [12]. The response to histamine was increased in isolated airways from patients with high levels of total serum IgE *in vivo* as compared with airways from patients with low IgE. The increase in responsiveness associated with IgE was comparable with the effect that could be achieved by incubation of isolated airways from patients with low serum IgE, with IgE-rich serum *in vitro*, a procedure known as passive sensitization. However, when airways taken from patients with high levels of IgE were

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**Table 1** Mean values  $\pm$  SEM of patients' characteristics

| Group of patients | Number (male) | Age (yr)   | Pack years | VC (L)          | FEV <sub>1</sub> (% pred.) | Total IgE U/mL <sup>†</sup> |
|-------------------|---------------|------------|------------|-----------------|----------------------------|-----------------------------|
| Non/ex-smokers    | 11 (11)       | 66 $\pm$ 1 | 45 $\pm$ 9 | 4.26 $\pm$ 0.20 | 104 $\pm$ 5                | 15 (1.9)                    |
| Current smokers   | 27 (22)       | 59 $\pm$ 2 | 50 $\pm$ 5 | 3.81 $\pm$ 0.19 | 78 $\pm$ 3*                | 24 (2.0)                    |

VC = vital capacity; FEV<sub>1</sub> = forced expiratory volume in 1 s; % pred. = % predicted [13]. Non-smokers = stopped smoking at least 2 years ago or had never smoked, current smokers = smoked until the week before surgery. Data of five patients from the non-smokers and current smokers had been included in the analysis of a prior study [12]. <sup>†</sup>Geometric mean and SEM expressed as factor. \* $P < 0.01$  as compared to non/ex-smokers.

passively sensitized, this did not lead to a further increase in histamine responsiveness. Since the results also suggested a potential influence of the patients' smoking status, we aimed in the present study to assess the effect of current smoking on airway reactivity *in vitro*. To exclude any effect of elevated serum IgE, only airways taken from patients with low serum IgE levels were included in the analysis.

## Methods

### Tissue preparation

Macroscopically normal bronchial tissue was obtained from patients undergoing surgery for lung cancer who showed serum IgE levels below 100 U/mL. Immediately after resection, peripheral airways (1–4 mm internal diameter) were dissected free of alveolar tissue and cut into rings (2–4 mm length). Tissues were rotated overnight at  $\approx 20^\circ\text{C}$  in tubes containing modified Krebs buffer (composition in mM: NaCl 118.4, KCl 4.7, MgSO<sub>4</sub> 0.6, CaCl<sub>2</sub> 1.3, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25.0, glucose 11.1) in the absence (non-sensitized controls) or presence of sensitizing serum (10% Vol./Vol.). Sensitizing sera were prepared from whole blood of individuals demonstrating high total IgE (250 to  $> 3000$  U/mL) and specific antibodies (FAST, fluorescent allerge sorbent test class,  $\geq 3$ ) against allergen (*Dermatophagoides farinae*). Sera were not pooled but were frozen individually in 200–250- $\mu\text{L}$  aliquots until required. On average the sera used in smokers and non-smokers did not differ in their IgE content.

The next morning bronchial rings were transferred to 10-ml organ baths containing oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) modified Krebs buffer (pH 7.4; 37  $^\circ\text{C}$ ). Tissues were equilibrated for about 60 min at a resting tension of 300–400 mg, before the commencement of experimental protocols.

### Tension measurements

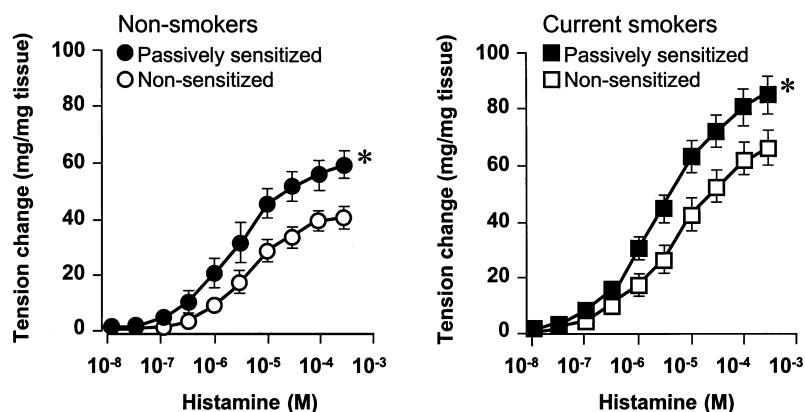
All responses were recorded as changes in isometric tension (mg). At the beginning of each experiment a single dose of the  $\beta$ -adrenoceptor agonist, isoprenaline (1  $\mu\text{M}$ ), was applied to determine the amount of inherent tone. After several washings and re-equilibration of the tissues, histamine concentration-effect curves within a range of 10 nM–0.3 mM were constructed. In some experiments histamine concentration-effect curves were followed by allergen (*D. farinae*) concentration-effect curves within a concentration range of 0.03–30 U/mL. All concentration-effect curves were obtained in a cumulative manner, using incremental concentrations spaced at half log<sub>10</sub> intervals. At the end of the experiments, tissues' wet weights were recorded.

### Measurements and analysis of results

All concentration-effect curves were acquired in at least one passively sensitized and one non-sensitized bronchial ring from the same donor. The potency of histamine was calculated from concentration-effect curves by non-linear curve fitting for each individual tissue and expressed as pEC<sub>50</sub>, i.e.  $-\log_{10}$  of the concentration of histamine giving the half-maximal effect. Tension changes were expressed in mg per mg tissue mass (mg/mg tissue). Histamine concentration-effect curves were compared between the different conditions (passively sensitized and non-sensitized; non-smoking, current smoking and current smoking with high IgE) using repeated measures analysis of variance (ANOVA), with the different conditions as between-factors and histamine concentration as within-factor.

To determine whether the results were influenced by the serum IgE levels, these levels were added in some analyses, either as covariates or as an additional binary factor. The two levels of this factor were chosen according to the individual's serum IgE, depending on the fact of whether it

**Fig. 1** Mean (SEM) histamine concentration-effect curves of airways without and after passive sensitization. Airways were taken from non-smokers (a) or smokers (b) with low serum IgE levels. Isometric tension changes are expressed as mg tension change per mg of tissues' wet weights. \* $P < 0.01$  according to the comparison of concentration-effect curves by ANOVA.



exceeded the median IgE or not. Whether curves did differ statistically was judged from the appropriate between-factors in ANOVA, as well as their interactions with the within-factor. Maximum contraction and  $pEC_{50}$ , as estimated from the histamine concentration-effect curves, were also compared with each other by ANOVA or paired or unpaired  $t$ -tests. Allergen responses were expressed as a percentage of the maximal responses to histamine (% hist. max.). In the analysis of allergen concentration-effect curves the same approach as for histamine was followed; however, we did not evaluate maximum contraction and  $pEC_{50}$ , since no complete concentration-effect curves were obtained. All values quoted are mean  $\pm$  SEM.  $P$ -values  $< 0.05$  were considered significant in ANOVA or two-tailed comparisons.

## Results

### Tissue donors

Experiments on isolated airways from 38 patients were analysed. Eleven patients were non-smokers (one never smoked, 10 were ex-smokers who had not been smoking for at least 2 years) and 27 current smokers (Table 1). There was no significant difference in serum IgE levels between the groups but current smokers showed a significantly ( $P < 0.01$ ) reduced forced expiratory volume in one second ( $FEV_1$ ) in L as well as percentage predicted [13] as compared with non-smokers.

### Baseline characteristics of bronchial rings

The number of individual ring preparations per experiment ranged between 2 and 10 passively sensitized and 1–4 non-sensitized tissues. The wet weight ( $10.60 \pm 0.37$  mg,  $n = 76$ ), resting tension ( $387 \pm 9$  mg) and inherent tone (i.e. the magnitude of relaxation after a single dose of isoprenaline:  $212 \pm 11$  mg) showed no statistically significant differences either between tissues from non-smokers

and current smokers or between passively sensitized and non-sensitized tissues.

### Responses to histamine

Histamine caused concentration-dependent contractions in all tissue preparations (Fig. 1). In non-sensitized airways from the current smokers, histamine responses were significantly increased as compared with non-sensitized airways from non-smokers (ANOVA,  $P < 0.001$ ; Figs 1a and b). The difference in histamine-response curves was reflected in a difference in maximal contractions but not  $pEC_{50}$  between both groups ( $P = 0.02$ ).

Passive sensitization of bronchi from non-smokers as well as current smokers significantly increased responsiveness to histamine, as compared with non-sensitized tissues from the same individuals (ANOVA,  $P < 0.001$  in both non-smokers and current smokers; Figs 1a and b). It led to a significant increase in maximal contraction in non-smokers ( $P = 0.015$ ) and a significant increase in both maximal contraction ( $P = 0.007$ ) and  $pEC_{50}$  ( $P = 0.005$ ) in current smokers with low IgE. Furthermore, maximal contractions but not values of  $pEC_{50}$  after sensitization differed significantly between groups ( $P = 0.01$ ).

A simultaneous comparison of bronchial rings from both groups under baseline conditions and conditions of passive sensitization demonstrated that the difference in baseline values and the effect of sensitization were purely additive. All interactions in ANOVA that would have demonstrated deviations from additive effects, were non-significant ( $P > 0.80$ ). When IgE was introduced either as a covariate or as an additional categorical factor in the comparison between non-smokers and smokers, it turned out to be non-significant, and the results of the analyses were essentially unchanged.

### Responses to allergen

In isolated airways from non-smokers or current smokers allergen-induced contractions were very weak (Figs 2a and

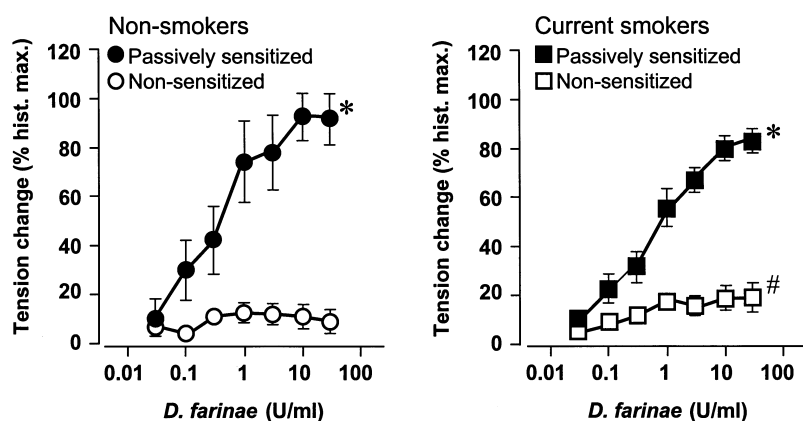


**Fig. 2** Mean (SEM) allergen concentration-effect curves of airways without and after passive sensitization. Airways from non-smokers (a) or smokers (b) with low serum IgE were used. Contractile responses to allergen are expressed as percentage of maximum histamine response. \* $P < 0.01$  according to the comparison of concentration-effect curves by ANOVA, # $P = 0.01$  regarding the dependence on allergen concentration (ANOVA).

b). They were not statistically significant from zero in non-smokers ( $n = 9$ ), in contrast to current smokers (ANOVA,  $P = 0.001$ ,  $n = 20$ ). Compared with each other, dose-response curves were not significantly different. As compared with non-sensitized control tissues, allergen (*D. farinae*) caused concentration-dependent contractions in passively sensitized tissues from non-smokers (ANOVA,  $P < 0.001$ ,  $n = 9$ ; Fig. 2a). The same was true in current smokers ( $P < 0.001$ , Fig. 2b). Allergen response curves after passive sensitization did not differ significantly between the two groups. When both groups were analysed with regard to the effects of passive sensitization and smoking simultaneously, they were not different in any respect.

#### Relationship between lung function and in vitro parameters

In none of the groups was there a statistically significant relationship between the physical characteristics of the patients, including lung function parameters ( $FEV_1/VC$  and  $FEV_1\%$ pred), and the parameters of airway responsiveness *in vitro*. When dose-effect curves were analysed in a repeated-measures ANOVA design including smoking status, sensitization and a binary variable indicating a deterioration in lung function as defined by  $FEV_1/VC < 70\%$ , still only smoking status and sensitization turned out to be significant determinants. This was also true for other definitions of airway obstruction such as  $FEV_1 < 80\%$  predicted.

#### Discussion

Our data demonstrate that smoking is an *in vivo* determinant for airway smooth muscle hyper-reactivity *in vitro* in patients with normal levels of serum IgE. Isolated airways from current smokers were hyper-responsive to histamine as compared with airways from non-smokers. Incubation with serum containing a high level of IgE, i.e.

passive sensitization, caused an increase in histamine responsiveness of a similar magnitude in both groups. These results suggest that smoking plays a role in the development of bronchial hyper-reactivity independently from IgE.

The concept underlying the present study evolved from a previous investigation addressing the role of serum IgE for airway hyper-responsiveness [12]. We showed that histamine responsiveness was increased in isolated airways from patients with high levels of serum IgE as compared with airways from patients with low IgE; similar associations between serum IgE levels and bronchial hyper-responsiveness are known from epidemiological data [7,8]. In accordance with epidemiological data demonstrating an association between IgE and smoking as well as atopy [14], most of the previously studied patients with high levels of IgE were current smokers; however, IgE and not smoking appeared to be the primary cause of airway hyper-responsiveness. In the present study we aimed to assess the effect of smoking separately, without interference from serum IgE.

According to our data active smoking until the time of surgery was associated with an increase in histamine responsiveness in isolated airways *ex vivo* as compared with airways from patients who were either ex-smokers or never smoked. These findings are in line with epidemiological observations showing a higher prevalence of bronchial responsiveness in current smokers than in non-smokers [9,15]. Since all patients included in our study had low levels of serum IgE, it is suggested that smoking *per se* is capable of inducing airway hyper-responsiveness independently from IgE.

Although the current smokers included in our study showed a significantly higher degree of airway obstruction than the ex-smokers, these differences did not correspond to differences in airway reactivity *in vitro*, and concentration-effect curves from patients with a ratio of  $FEV_1$  to  $VC < 70\%$  [16] were not different from those of patients

with values = 70%. Until now, a number of studies have investigated *in vitro* reactivity in patients with COPD [16–19]. De Jongste and co-workers compared patients with COPD and patients without COPD regardless of their smoking status and IgE levels [16]. They observed an increased histamine responsiveness, assessed as maximal isometric force generation in mg/mg tissue weight, in isolated airways from patients with COPD. Two other studies performed in current smokers with and without airway obstruction did not find such a relationship between *in vivo* and *in vitro* reactivity [17,18]. Again, however, IgE levels were not taken into consideration and there was no comparison with airways from non-smokers. In contrast, Vincenc and co-workers reported on *in vitro* data obtained in six current smokers and eight never-smokers or ex-smokers, but did not either analyse the data with respect to smoking nor report how long ago the ex-smokers had been required to have stopped smoking [20]. Therefore, studies on *in vitro* reactivity in COPD appear to be inconclusive. In addition, it should be taken into consideration that all studies based on lung resection material, including our own, are inevitably subjected to the limitation that the localization of the lung tumour might have affected lung function in single patients.

Passive sensitization, i.e. the incubation of tissue preparations with IgE-rich serum from atopic patients, led to an increase in non-specific responsiveness to histamine *in vitro*. As shown previously, this effect is likely to be caused by factors in the serum of atopic individuals that appear to be associated with IgE, but are not IgE itself [21–23]. However, these factors are unknown until now. Since IgE synthesis is regulated by Th2-type cytokines and Th2-type cytokines can alter airway reactivity and smooth muscle synthetic function [24–26], it seems reasonable to assume that the factors are to be found among these cytokines.

On the first sight, there seems to be a mechanistic link between the increases in histamine responsiveness associated with smoking *in vivo* and passive sensitization *in vitro*. Smoking stimulates Th2-type (IL-4) cytokine production and complementary Th2-type cytokines play a key role in the regulation of serum IgE levels [27–31]. Therefore, it might be hypothesized that the unknown serum factors associated with high IgE levels in atopy, were also active in the smokers, leading to enhanced histamine responsiveness of their isolated airways *in vitro*.

However, this hypothesis appears to be justified only as far as smoking is accompanied by elevated IgE levels, which normalize after cessation of smoking [8,10,32,33]. If the serum factor was regularly associated with high IgE levels, we would expect that the smokers, who showed increased responsiveness, also showed elevated serum IgE levels; in contrast, in our study they showed low levels.

Secondly, passive sensitization increased histamine responses in smokers as well as non-smokers with low serum IgE to a similar degree as reflected in the parallel upward shift of the concentration-effect curves (see Fig. 2). This suggests that the effects of passive sensitization *in vitro* and those of smoking *in vivo* are based on additive and independent mechanisms.

The procedure of passive sensitization has been introduced to transfer specific responsiveness to allergen from atopic to non-atopic individuals [34]. Previous studies revealed that the induction of allergen responses depends on the presence of IgE in the serum used for sensitization. Serum depleted from IgE is not capable of inducing allergen responsiveness [22], whereas monoclonal IgE does [21]. In the present study histamine responses were increased in airways from smokers and could be further enhanced by passive sensitization above the level achieved in passively sensitized tissues from non-smokers. In contrast, the pattern of allergen responses was different; responses were very similar in airways from smokers and non-smokers, not only under control conditions but also after passive sensitization. The increase in allergen-induced contractions to about the same level (Fig. 2) might be interpreted as a direct consequence of the IgE within the sensitizing serum. Notably, the presence of a weak baseline response to allergen is in accordance with epidemiological results indicating a higher rate of sensitization against house dust mite in smokers [35].

Our findings indicate, and confirm our earlier findings, that the pathways mediating allergen responses are not identical to those mediating histamine responses and that the induction of specific responses to allergen is independent from that of non-specific responses to histamine. This assumption is supported by studies showing that the intravenous application of anti-IgE antibodies greatly inhibited early and late phase responses in patients with allergic asthma [36,37], but did not affect non-specific bronchial responsiveness to a clinically relevant extent [36], in line with *in vitro* data [38].

In addition, our data suggest that smoking leads to non-specific airway hyper-responsiveness through mechanisms that are different from those enhancing histamine responses within the model of passive sensitization. There is evidence that the induction of hyper-responsiveness by passive sensitization involves alterations within the smooth muscle [5,39–41], including an increase in the content of myosin light chain kinase [42,43], changes in membrane potential, and/or alterations in smooth muscle phenotype [44] and secretory function [26,45]. The impairment in lung function as found in smokers but not in non-smokers (Table 1), as well as data from the literature [6], are in favour of the hypothesis that at least partially irreversible, long-term structural and functional alterations within the bronchial

tissue or airway smooth muscle are major factors that contribute to airway hyper-responsiveness in smokers. Most recently it has been suggested that airway hyper-responsiveness in patients with COPD, who are current smokers, is primarily determined by alterations within the airways rather than by the altered mechanical load caused by the destruction of lung parenchyma [46].

In conclusion, our study demonstrates that smoking is associated with non-specific airway hyper-responsiveness of human airways *ex vivo*, at low serum IgE levels. The mechanism through which smoking leads to hyper-responsiveness appears to be different from that which induces hyper-responsiveness in the process of passive sensitization, and is not associated with IgE. In contrast, the development of specific responsiveness to allergen seems to depend predominantly on IgE and is independent from that of non-specific responsiveness.

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