

BRIEF COMMUNICATIONS

Corticosteroid treatment selectively decreases mast cells in the smooth muscle and epithelium of asthmatic bronchi

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Abstract

Background: Mast cells are important in the pathophysiology of airway inflammation and evidence suggests their sub-localisation within the airway is altered in asthma. Little is known about the effect of corticosteroids on mast cell localisation within the bronchi.

Methods: We therefore performed an immunohistochemical analysis of mast cell numbers within the smooth muscle, epithelium and submucosa of healthy subjects ($n = 10$) and well-characterised asthmatic patients, using either β_2 -agonists alone ($n = 10$) or β_2 -agonists and inhaled corticosteroids ($n = 10$).

Results: Patients using inhaled corticosteroids displayed significantly lower numbers of mast cells within their epithelium and smooth muscle compared to those not treated with inhaled corticosteroids. Submucosal mast cells were not affected by corticosteroid treatment. Numbers of smooth muscle mast cells correlated with bronchial responsiveness and epithelial mast cells with exhaled NO.

Conclusion: We demonstrate that glucocorticosteroids differentially affect mast cell numbers within specific airway sub-locations highlighting the importance of mast cell and smooth muscle/epithelial interactions in asthma pathogenesis.

Asthma is a chronic inflammatory disorder of the airways involving several cell types. Mast cells are important as they drive the IgE-mediated allergic reaction, respond to a variety of stimuli and produce an array of preformed granule proteins and newly synthesised mediators (1). Recent results have demonstrated that mast cells within the airways of subjects with asthma show a different pattern of localisation compared to healthy controls, differences which could be associated with asthma pathogenesis. Increased numbers of mast cells are found both in the airway smooth muscle of patients with asthma (2, 3) and within the epithelial layer (4, 5). However, the effect of inhaled corticosteroid treatment on the distribution of mast cells within the airway is unknown. Our aim was therefore to examine the effect of inhaled corticosteroid treatment in patients with mild asthma

on mast cell numbers within three locations in the bronchi: the epithelium, submucosa and smooth muscle.

Bronchial biopsies were obtained by flexible fiberoptic bronchoscopy in accordance with ERS/ATS guidelines from 10 healthy volunteers and 20 subjects with intermittent-to-mild asthma according to GINA criteria. Ten of these asthmatic subjects used short-acting β_2 -agonists as their only medication, whereas ten used both short-acting β_2 -agonists and inhaled corticosteroids (400–1200 μ g/day budesonide or eq.). All subjects have been described previously in more detail (6). This study was approved by the Ethics Committee of Karolinska University Hospital (00-267), and subjects provided written consent.

Biopsy specimens were embedded in glycol methacrylate as previously described (7). The primary antibodies used were

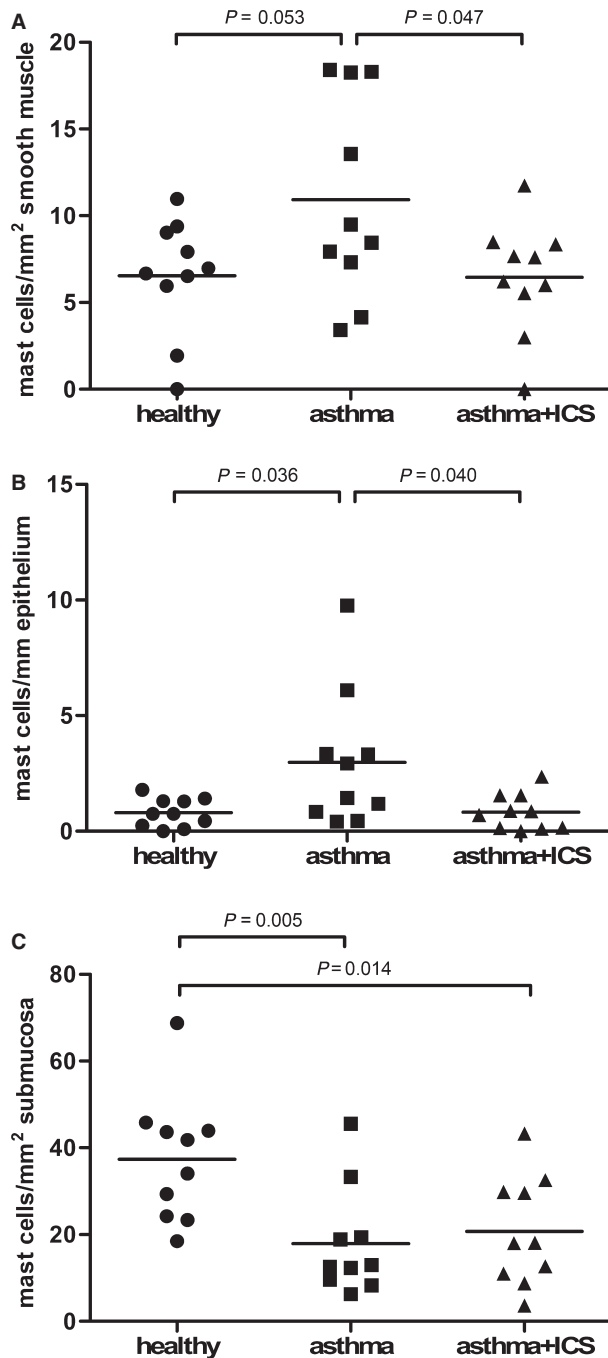


Figure 1 Mast cell numbers within the smooth muscle (A), epithelium (B) and submucosa (C) in bronchial biopsies taken from healthy controls, asthmatics not using inhaled corticosteroids and asthmatics using inhaled corticosteroids. Results are presented as individual data points with mean bars. Data were normally distributed and compared using Student's *t*-test for unpaired samples.

mouse anti-human smooth muscle actin and mouse anti-human mast cell tryptase, and clone AA1 (DAKO Cytomation, Glostrup, Denmark). On average, 4.5 biopsies were analysed per patient, and total areas of smooth muscle

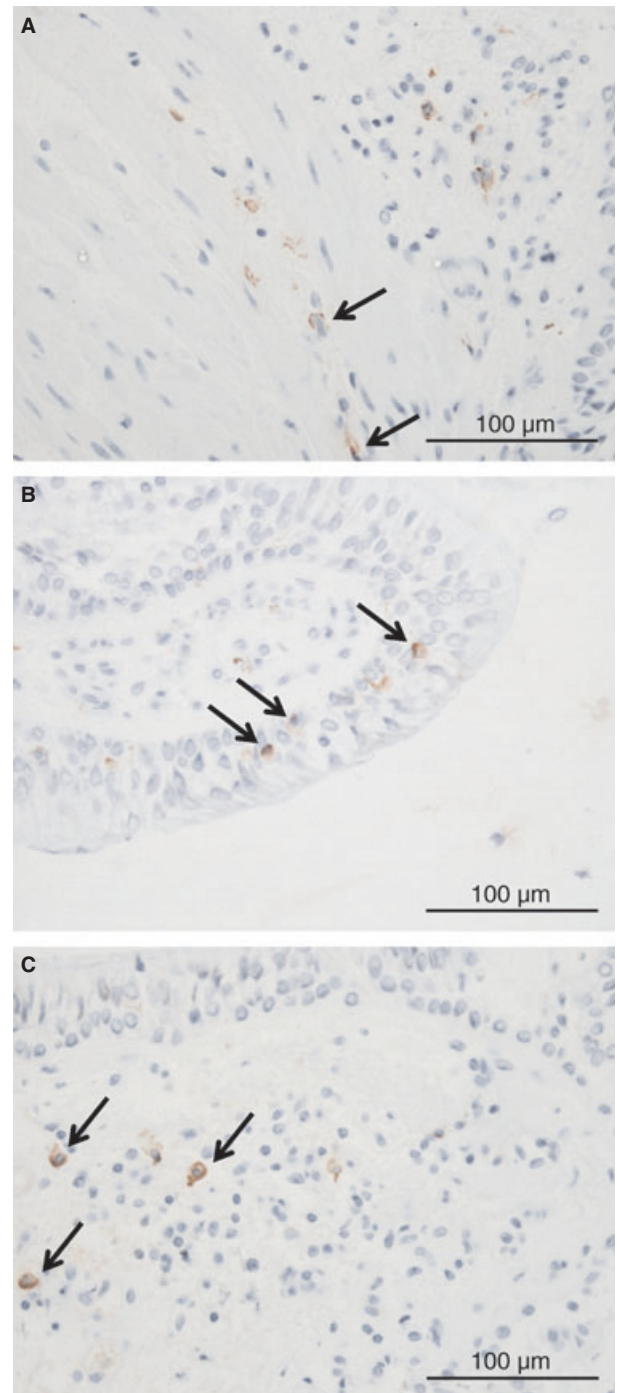


Figure 2 Representative photomicrographs showing mast cells within different bronchial biopsy sub-locations. Arrows show red tryptase-positive mast cells within the smooth muscle (A), epithelium (B) and submucosa (C). Nuclei were counterstained using Mayer's haematoxylin and scale is shown using 100-µm markers.

(1.3 mm²) and submucosa (1.0 mm²), and basement membrane length (4.1 mm) were measured by image analysis using a Leica DM RXA microscope with Leica Qwin 2.7

Table 1 Correlations between mast cell numbers and clinical characteristics of asthmatic subjects

	Smooth muscle mast cells	Submucosal mast cells	Epithelial mast cells
Methacholine PD ₂₀ (log µg)	$\rho = 0.6, p = 0.008$	NS	NS
NO (ppb)	$\rho = 0.6, p = 0.018$	NS	$\rho = 0.8, p < 0.001$

software (Leica Microsystems, Wetzlar, Germany). Smooth muscle area was only assessed in biopsies with detectable actin staining, and only the basement membrane length of intact, nondamaged epithelium was measured. Nucleated AA1 mast cell tryptase-positive cells within these areas were counted. Samples were coded so that microscopists were not aware of subjects' grouping or clinical diagnosis.

We found greater numbers of mast cells/mm² of smooth muscle in asthmatic subjects (10.9 ± 5.8) compared to healthy volunteers (6.5 ± 3.3 , $P = 0.053$) and asthmatic subjects using inhaled corticosteroids (6.5 ± 3.2 , $P = 0.047$) (Fig. 1A). Similarly, epithelial mast cell numbers were greater in asthmatic subjects (3.0 ± 3.0) compared to healthy subjects (0.8 ± 0.6 , $P = 0.036$) and asthmatics using inhaled corticosteroids (0.8 ± 0.8 , $P = 0.040$) (Fig. 1B). These findings confirm previous observations of increased epithelial and smooth muscle mast cells in asthma (2–5). The mechanism underlying reduced numbers of mast cells following steroid treatment is unknown but may involve a reduction in epithelial and smooth muscle-derived chemotactic factors including stem cell factor, a mast cell survival factor (8, 9). Representative photomicrographs showing mast cells within the smooth muscle, epithelium and smooth muscle are shown in Fig. 2.

In contrast, submucosal mast cells were not affected by corticosteroid treatment and unexpectedly, numbers were higher in controls (37.4 ± 14.8) compared to asthmatics (17.9 ± 12.4 , $P = 0.005$) (Fig. 1C). The reason for this finding remains unknown and difficult to explain, although previous immunohistochemical analyses of submucosal mast cells in asthmatics have shown variable results, with several reports showing unchanged numbers (3, 5, 10), whereas others show increased submucosal mast cells (4). Corticosteroids are also reported to be both with and without effect on submucosal mast cells (4, 7, 11–14). Furthermore, the subjects in the present study were well characterised with current documentation of bronchial responsiveness (6). Interestingly, Balzar et al. (4) recently showed that mild asthma was associated with higher numbers of total submucosal mast cells than more severe disease. Along with the fact that in our study, when all subjects (including healthy controls) were grouped together, submucosal mast cell numbers showed a positive correlation with FEV₁% predicted ($\rho = 0.4$; $P = 0.02$), that is, more submucosal mast cells = better lung function, we speculate that in our study

the submucosa may have been a more 'normal' location for bronchial mast cells.

Smooth muscle mast cell numbers correlated with bronchial responsiveness to methacholine, suggesting that the co-localisation of these two cell types is particularly relevant to the mechanisms involved in BHR (Table 1). In accordance, several mast cell mediators including tryptase, IL-4 and IL-13 are known to affect bronchial responsiveness (1). Exhaled nitric oxide (NO) is thought to reflect airway inflammation and here showed a particularly strong correlation with epithelial mast cell numbers (Table 1). The source of the increased NO in asthma has been debated. Our finding supports the possibility that epithelial mast cells somehow contribute to the increased expression or activity of NO synthases under inflammatory conditions (15).

The finding that corticosteroid treatment affects mainly epithelial and smooth muscle mast cells may be explained by the presence of different mast cell phenotypes within airway locations, possibly differing with regard to steroid sensitivity. It is known that multiple site-specific mast cell subpopulations exist in the lung, differing with regard to expression of proteases, FcεR1α, renin, cytokines, growth factors and leukotriene pathway enzymes (16). Although little is known about the steroid sensitivity of mast cell subtypes, it was recently shown that markers of intraepithelial mast cells specific to Th2-high asthma correlated with lung function improvement after ICS therapy (17); however, the exact mast cell phenotypes and their sensitivity to corticosteroids in different airway locations remain to be examined.

In summary, we confirm that mast cell numbers are increased within the epithelium and smooth muscle in subjects with asthma, and we also demonstrate that inhaled corticosteroids are associated with lower mast cell numbers in these locations. Submucosal mast cell numbers, however, were not affected by corticosteroid treatment. Smooth muscle mast cells correlated with bronchial responsiveness and mast cell numbers within the epithelium correlated strongly with exhaled NO. These findings highlight the importance of specific interactions between mast cells with the airway smooth muscle and epithelium, and demonstrate how mast cells within specific sub-locations may respond differently to glucocorticosteroids.

Authors' contributions

All authors were involved in the preparation of the article and gave their final approval of the submitted version. AJ performed all data analysis and carried out immunohistochemical analyses. PG recruited and performed clinical assessments of the patients included. EH prepared all bronchial biopsy material. MA, SED, GN and BD all contributed to the initiation and design of the study.

Conflict of interest

None of the authors of this article have any conflict of interest to disclose.

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