Mast cell-associated alveolar inflammation in patients with atopic uncontrolled asthma

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MAST CELL-ASSOCIATED ALVEOLAR INFLAMMATION IN PATIENTS WITH ATOPIC UNCONTROLLED ASTHMA.

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ABSTRACT

Background: A significant proportion of patients with asthma have persistent symptoms despite treatment with inhaled glucocorticosteroids (ICS).

Objective: We hypothesized that in these patients the alveolar parenchyma is subjected to mast cell-associated alterations.

Methods: Bronchial and transbronchial biopsies from healthy controls (n=8), patients with allergic rhinitis (AR) (n=8) and patients with atopic uncontrolled asthma (symptoms despite treatment with ICS: mean dose: 743 μg/day, n=14) were processed for immunohistochemical identification of mast cell subtypes and mast cell expression of FcεRI and surface-bound IgE.

Results: Whereas no difference in density of total bronchial mast cells was observed between asthmatic patients and healthy controls, the total alveolar mast cell density was increased in the asthmatics ($p<0.01$). Division into mast cell subtypes revealed that in bronchi of asthmatics, MC_T numbers decreased compared to controls ($p\leq0.05$), while MC_{TC} increased ($p\leq0.05$). In the alveolar parenchyma from asthmatics an increased density was found for both MC_T ($p\leq0.05$) and MC_{TC} ($p\leq0.05$). The increased alveolar mast cell densities were paralleled by an increased mast cell expression of FcεRI ($p<0.001$) compared to the controls. The asthmatics also had increased numbers ($p<0.001$) and proportion ($p<0.001$) of alveolar mast cells with surface-bound IgE. Similar increases in densities, FcεRI expression, and surface-bound IgE were not seen in separate explorations of alveolar mast cells in patients with AR.

Conclusions: Our data suggest that patients with atopic uncontrolled asthma have an increased parenchymal infiltration of MC_T and MC_{TC} populations with increased expression of FcεRI and surface-bound IgE compared to atopic and non-atopic controls.

Clinical Implications: The present mast cell alterations in the alveolar parenchyma represent a novel feature of asthma that may have clinical implications and support the rational to target the distal airways in uncontrolled asthmatics on ICS.

Capsule Summary: This study demonstrates that in asthmatic patients with persistent symptoms despite conventional ICS therapy the alveolar parenchyma is infiltrated by increased numbers of mast cells that have increased expression of the high-affinity receptor for IgE (FcεRI).

Key words: mast cells; asthma; FcεRI; IgE; allergy; peripheral inflammation; alveolar parenchyma.

Abbreviations

IgE: immunoglobulin E

FcεRI: high affinity IgE receptor

ICS: inhaled glucocorticosteroids

GINA: global initiative for asthma

COPD: chronic obstructive pulmonary fibrosis

CF: cystic fibrosis

IPF: Idiopathic pulmonary fibrosis

AR: allergic rhinitis

ACT: asthma control test

MC_{TC}: tryptase and chymase positive mast cells (connective tissue mast cells)

MC_T: tryptase positive mast cells (mucosal mast cells)

HRP: horseradish peroxidase

DAB: 3,3' diaminobenzidine

AP: alkaline peroxidase

PD_{20}: cumulative dose of bronchoconstrictor where FEV_1 fell by 20 % or more

FEV_1: forced expiratory volume in 1 second

FVC: forced vital capacity

p.r.n: pro re nata, as needed

INTRODUCTION

Asthma is a chronic inflammatory airway disease that is characterized by a reversible airway obstruction and airway hyperreactivity[1, 2]. Most patients have an allergic component where the immunoglobulin E (IgE) plays a central role by activating key immune cells through the high affinity IgE receptor, FcεRI[3, 4]. Although treatment with bronchodilators and inhaled glucocorticosteroids (ICS) generally provide good control of the disease, a significant proportion of the asthma patients have persistent symptoms despite conventional therapy[5]. This phenomenon, referred to as uncontrolled asthma[6,7], represents a major challenge for improved asthma control.

Despite the clinical significance of uncontrolled asthma, little is known about the inflammatory processes that evoke symptoms in this group of patients. One possibility is existence of steroid-resistant inflammatory components in the central airways[8]. Another alternative is involvement of peripheral airways[9, 10], which are difficult to reach by conventional inhalation therapy[11]. The few previous studies that have explored transbronchial biopsies from asthmatics provide clear indications that both small airways and alveolar tissues may be subjected to a cellular inflammation in asthma[12-14].

Mast cells have long been recognized as a key cell of the allergic reaction in atopic asthma, by virtue of their expression of FcεRI[15]. They are widely present in high numbers in human peripheral airways, including the alveolar region[10, 12, 16-18]. We recently identified a distinct mast cell population in the alveolar tissue of normal lungs[19]. These poorly studied alveolar mast cells, which are characterized by a low FcεRI expression, comprise a large mast cell population in the human lung[12, 19, 20].

Increased numbers of bronchial mast cells and elevated levels of IgE have been described in allergic asthma[10, 12, 17, 21, 22]. From this, and the fact that high IgE-levels may lead to increased FcεRI expression on mast cells[23, 24], we hypothesized that patients with ICS-treated, atopic uncontrolled asthma have a significantly altered mast cell population where the normally FcεRI low-expressing alveolar mast cells have acquired an FcεRI-expressing phenotype. To test this hypothesis mast cell densities and mast cell expression of FcεRI and surface-bound IgE were analyzed in bronchial and transbronchial biopsies from atopic, uncontrolled asthmatics and healthy control subjects. Separate comparisons were also made with patients with atopic allergic rhinitis (AR) with no concomitant asthma.

METHODS

Subjects

Patients with atopic uncontrolled asthma, non-atopic and atopic control groups:

The present study involved 14 non-smoking patients with uncontrolled atopic asthma according to GINA guidelines and asthma control test (ACT)[6, 25]. Eight healthy never-smoking non-atopic subjects that had negative skin prick test (SPT), were not hyper-responsive to metacholine, and lacked of any history of respiratory symptoms were used as controls. As a separate control group, representing atopy without asthma, included 8 patients with clinically confirmed AR[26].

From each of the 30 subjects, 5 central airway biopsies and 5 transbronchial biopsies (in total 300 biopsies) were collected during a study period from November 2005 to June 2010 at the Department of Respiratory Medicine, Lund University Hospital (for methodological details, see ref[27] and online supplement). All subjects gave their written informed consent to participate in the study, which was approved by the ethics committee in Lund (LU412-03).

Lungs from patients with advanced stages of non-atopic patients with chronic obstructive pulmonary disease (COPD) and cystic fibrosis were included to study the FcεRI expression on alveolar mast cells in lungs subjected to a non-allergic inflammation. The clinical characterization of these patients and their matching controls, are presented in the online supplement and Table E2.

Allergy screening

Standardized skin prick test (SPT) (Alk Abello, Copenhagen, Denmark) was performed on all subjects in all cohorts (controls, asthma, AR, COPD and CF) and was used to screen for sensitization for 10 aeroallergens (birch, timothy, mugwort, cat, dog, horse, D. pteronyssinus, D. farinae, Aspergillus fumigatus and Cladosporium herbarum). Atopy was defined as a positive SPT (weal reaction larger or equal to histamine positive control) to one or more allergens. Patients with positive SPT to pollen without any other sensitivity were classified as seasonal, whereas patients with multiple sensitivities (pollen, animal, mould and/or mite) were classified as perennial. For all subjects with positive SPT to seasonal pollen, bronchoscopy procedures were performed outside pollen season.

Tissue Processing

Bronchial and Transbronchial biopsies

All biopsies from uncontrolled asthmatics and 4 out of 5 bronchial and 4 out of 5 transbronchial biopsies from the healthy controls were immediately placed in 4% buffered formaldehyde, dehydrated, embedded in paraffin. Serial sections from all paraffin blocks were stained with Mayer's haematoxylin and these were used to select 2 bronchial and 2 transbronchial biopsies from each patient that had a well-preserved morphology and were without any crush, or mechanically-induced stretch artifacts. The selected biopsies were used for quantification of mast cell-related parameters.

The remaining biopsies from the control patients and biopsies the rhinitis cohort were immersed in periodate-lysine containing 1% paraformaldehyde (1% PLP) for 4 h at 4°C. Specimens were embedded in OCT (Tissue-Tek, Miles Laboratories, IN), and frozen. Serial cryo sections from all biopsies were generated and stored until histological assessments (see below).

Immunohistochemistry

All antibodies used have been extensively validated for staining of paraffin embedded human tissue sections (and cryo sections) in research and routine clinical diagnosis (Table E1). For details on immunohistochemical protocols and specificity controls[19, 20], see online supplement). Staining was absent in sections using isotype-matched control antibodies (Dako, Glostrup, Denmark). Staining was performed identically for all patient groups.

Double Immunohistochemical Staining of MC_{TC} and MC_T

A double staining protocol was used for simultaneous visualization of MC_{TC} and MC_T cells[18-20, 28-32]. The immunohistochemistry protocol was performed using an automated immunohistochemistry robot (Autostainer, Dako) with EnVision™ G|2 Doublestain System (K5361, Dako). For protocol details, see Table E1 and online supplement.

Immunohistochemical Identification of $Fc\varepsilon RI^+$ and IgE^+ Mast Cells

A triple staining immunofluorescence protocol[19, 20, 33, 34] was used to simultaneously visualize both MC_{TC} and MC_T populations together with their expression of the IgE receptor (FcεRI) or surface-bound IgE (see online supplement and Table E1).

Histological Analysis and Quantification

Quantification of Densities of Mast Cell Subtypes

High-resolution images of sections -stained for MC_{TC} and MC_T were generated through a 20x microscope lens by an automated digital slide-scanning robot (Scanscope CS™, Aperio, Vista, CA). In bronchial biopsies the densities of MC_T and MC_{TC} was also calculated in sub-anatomical compartments: *epithelium, subepithelial tissue (excluding smooth muscle and glands), smooth muscle tissue and subepithelial glands*. An image analysis program

(ImageScope, v10.0.36.1805, Aperio) calculated the tissue area within the delineated region by automatically excluding any non-tissue regions (i.e. regions without any tissue components) and the proper tissue density (expressed as cells / mm^2 tissue) was calculated for each mast cell subtype[14, 19, 20, 30, 35-37].

Quantification of FcεRI$^+$ and IgE$^+$ Mast Cells

After triple immunofluorescence staining, the filter setting was adjusted to reveal the tryptase-positive mast cells at 488 nm. By alternating the filter settings each tryptase-positive cell was examined for presence of chymase (647 nm) as well as expression of FcεRIα or surface-bound IgE (555 nm). The density of mast cells expressing FcεRI and IgE was calculated by multiply the percentage of $MC^{Fc_\varepsilon RI+}$ or MC^{IgE+} with the total mast cell density in the same tissue region (for further details and quantification on COPD and CF, see ref [18] and online supplement).

Statistical Analysis

Data were analyzed statistically on mean values from each patient, using Mann-Whitney rank sum test for comparison between two groups (disease vs. control) using GraphPad Prism v. 5 (GraphPad Software Inc., La Jolla, CA). For all outcomes, a p-value ≤ 0.05 was considered significant (* denotes $p \leq 0.05$, ** $p < 0.01$ and *** < 0.001).

RESULTS

Clinical Characteristics

An overview of the patient characteristics is presented in Table 1.

Uncontrolled asthma: The 14 asthma patients included in the study had symptomatic uncontrolled asthma (ACT score ranging from 11 to 21). All were atopic (i.e. positive SPT), and all but one had rhinitis. All asthma patients were treated with inhaled glucocorticosteroids (Budesonid) and inhaled bronchodilators (Table 1). Two patients were treated with leukotriene-receptor antagonists and 3 had nasal corticosteroids and anti-histamines p.r.n. In addition, 1 patient was treated for hypertension (losartan potassium/hydrochlorothiazide), 2 for gastritis (omeprazole) and 1 with vitamin B substitute. None of the patients were treated with anti-IgE therapy. Two patients had seasonal and 12 had perennial allergy. *AR:* The 8 patients with AR with no concomitant asthma all had positive SPT and were not hyper-responsive to metacholine (PD20 > 2000 μg). None of the AR patients were treated with inhaled bronchodilators or glucocorticosteroids. Two had nasal corticosteroids and 4 had anti-histamines p.r.n. One patient had seasonal and 7 had perennial allergy. *Healthy controls:* All healthy controls were without any respiratory symptoms, had normal lung function, and negative SPT and metacholine challenge test (PD20 > 2000 μg). FEV_1 % predicted was lower in patients with asthma (81 [63-108] FEV_1 % pred.) compared to healthy controls (98 [72-116] FEV_1 % pred., p = 0.03) and patients with rhinitis (107 [96-138] FEV_1 % pred., p = 0.001). No difference in FEV_1 % pred. was found between controls and rhinitis (p = 0.3). In addition, 5 CF and 10 COPD patients were investigated[19, 20] (for patient and protocol details, see online supplement).

Characterization of Mast Cell Phenotypes in Uncontrolled Asthma and Healthy Controls

Densities of MC_T and MC_{TC} Populations

In central airways, the total tissue density of mast cells did not differ between patients with uncontrolled asthma and healthy control subjects (Table 2). In contrast, the alveolar parenchyma displayed increased numbers of mast cells in patients with uncontrolled asthma compared to healthy controls (Table 2). The unaltered total mast cell numbers in central airways in uncontrolled asthma was a result of a decrease in MC_T numbers combined with an increase in MC_{TC} numbers compared to healthy controls (Table 2 and Figure 1). The significant increase in total alveolar mast cell numbers in uncontrolled asthmatics was due to an increase in both MC_T cells and MC_{TC} numbers compared to healthy controls (Table 2 and Figure 1).

Microlocalization of Mast Cell Subtypes in Central Airways

The highest density of mast cells was found in the lamina propria for both control subjects and asthmatics. No difference in the distribution of mast cells was found for the MC_T subclass in asthmatics compared to controls (Table E3). The MC_{TC} density increased in the smooth muscle layer in asthmatics (7.1 [0-25] mast cells per mm^2) compared to controls (1.0 [0-5] mast cells per mm^2, $p = 0.01$) (Table E3).

Expression of FcεRIα and IgE on Bronchial and Alveolar Mast Cells

Both FcεRIα and IgE immunoreactivity displayed a characteristic membrane staining in triple-stained immunofluorescence sections. As previously shown[12, 19, 38], the proportion of mast cell expressing FcεRIα was high in central airways in healthy subjects, and no significant difference in expression to uncontrolled asthmatics was observed (Figure 2A and

Table 2). The mast cell expression of FcεRIα did not differ between controls and asthmatics, neither for MC_T (p = 0.4) nor for the MC_{TC} subtype (p = 0.5). In contrast, in the alveolar parenchyma, the mast cell expression of FcεRIα was low in healthy controls and significantly higher in uncontrolled asthma (Figure 2B, C-D and Table 2). The increased FcεRIα expression in uncontrolled asthma was further confirmed using a computerized image analysis approach was used to calculate the area of FcεRIα immunoreactivity on individual mast cells (see online supplement).

In central airways, the proportion of IgE^+ mast cells was low in healthy controls and significantly increased in uncontrolled asthmatics (Figure 2E and Table 2). Also in the alveolar parenchyma, the proportion of IgE^+ mast cells was low in controls and significantly increased in the alveolar parenchyma (Figure 2F, G-H and Table 2). As for the expression of FcεRIα, no difference in mast cell-bound IgE was found between MC_T and MC_{TC} subclasses, neither in central airways (controls: p = 0.5, asthma: p = 0.2) nor in alveolar parenchyma (controls: p = 0.4, asthma: p = 0.1).

In alveolar parenchyma, the tissue density of FcεRIα positive mast cells was increased in uncontrolled asthmatics (132 [9-591] $MC^{Fc_\varepsilon RI_\alpha +}/mm^2$) compared to controls (1.5 [0-27] $MC^{Fc_\varepsilon RI_\alpha +}/mm^2$, p = 0.0003) (Figure 3A). Also an increase in the density of mast cells positive for surface-bound IgE was found in the asthmatics (133 [8-591] MC^{IgE+}/mm^2) compared to controls (0 [0-3] MC^{IgE+}/mm^2, p = 0.0001) (Figure 3B).

FcεRIα and IgE expression on Alveolar Mast Cells in AR and Non-Allergic Inflammatory Diseases

Allergic Rhinitis

283 In AR, no significant change in total mast cell numbers or the density of MC_T and MC_{TC} was
284 found in central airways or in alveolar parenchyma compared to healthy controls (Table 2).
285 No increase in the proportion of mast cells expressing the FcεRIα could be found in central
286 airways or in the alveolar parenchyma in patients with AR compared to controls (Table 2). No
287 significant increase in the proportion of mast cells with surface bound IgE was found in
288 central airways. However, an increase in the proportion of mast cells with surface bound IgE
289 was found in alveolar parenchyma in patients with AR compared to controls (Table 2).
290
291 *Non-Allergic Lung Diseases: Comparison to COPD and CF*
292 The FcεRIα expression was high in central airways in controls compared to the same
293 compartment in COPD and CF. In alveolar parenchyma, the mast cell FcεRIα expression was
294 low in controls; no significant change was found in COPD and CF patients (see online
295 supplement).
296

DISCUSSION

Accumulated evidence from physiological studies and tissue explorations suggest that inflammatory processes in the distal airways contribute to asthma pathogenesis[39]. The present study advances our insight about the nature of this inflammation by identifying an alveolar infiltration of altered MC_T and MC_{TC} populations as a novel histopathological feature.

The present study took advantage of our possibility to obtain bronchial as well as transbronchial biopsies, not only from patients with uncontrolled asthma, but also from healthy control subjects. Thus, our approach allowed the first exploration of how mast cells in both bronchial and alveolar compartments in uncontrolled asthmatics differ from healthy base-line conditions.

The discovery of increased FcεRI-expression on alveolar mast cells in uncontrolled asthma represents a major finding in this study. Mast cells in most types of tissues, especially those facing the external environment, have a high basal expression of FcεRI[15]. The alveolar mast cells, however, have under healthy conditions a very low FcεRI expression[19]. In this study the number of FcεRI expressing mast cells in the alveolar parenchyma in uncontrolled asthma was 40-fold higher than what could be observed in healthy controls. Furthermore, this was associated with an equally robust (500-fold) increase in numbers of mast cells with surface-bound IgE, suggesting that the alveolar mast cells in uncontrolled asthma have acquired a phenotype fully capable to classical IgE-mediated activation. Importantly, this feature seems to be specific to asthma since we by same staining techniques could not detect similar changes in patients with severe non-allergic alveolar inflammation (end-stage CF, or COPD) as well as atopic patients with AR.

The low expression of FcεRIα on alveolar mast cells in AR, present in our study, indicates that atopy *per se* does not cause the altered alveolar mast cell phenotypes found in uncontrolled asthma. It should however be noted that the situation may be different in AR patients during episodes of increased allergen exposure. Although we could not find increased FcεRI expression in central airways, previous studies have reported increased numbers of bronchial FcεRI^{+} mast cells in atopic, non-asthmatic subjects[38]. The vast majority of patients with asthma have rhinitis[42] and rhinitis, especially at severe stages, is a major risk factor for developing asthma[43-45]. Hence, in future studies it seems important to explore the FcεRI expression on alveolar mast cells in high-risk rhinitis patients and newly diagnosed asthma patients with rhinitis. If high FcεRI and IgE-expressing alveolar mast cells are present in additional asthma cohorts needs to be further investigated. Furthermore, we could not detect similar changes in other airway diseases characterized by extensive alveolar inflammation and/or remodeling like COPD and cystic fibrosis, despite a rich occurrence of alveolar mast cells in these diseases. It should however be noted that CF and COPD differs from the uncontrolled asthmatic group, not only in pathological features but also in treatment. Despite their medication, these patients have a significant remaining inflammatory response in the alveolar parenchyma. In this inflammation the alveolar mast cells have a low expression of the IgER.

Although most of the inhaled allergens are deposited in the conducting airways, common allergens may well be transported by respirable particles all the way to the alveolar region[46-48]. For patients sensitized to systemic allergens, the presence of FcεRI^{+} mast cells in the alveolar parenchyma could theoretically contribute to the increased risk of anaphylaxis associated with asthma[49]. If occurring, an IgE-driven allergic inflammation in the alveolar

parenchyma is likely to have pathophysiological implications. Several mast cell mediators have pro-fibrotic and matrix-modulating properties and may thus contribute to the structural alterations that recently have been observed in alveolar tissues from asthmatics[27].

In 2002, Brightling et al[17] showed that the density of mast cells in the airway smooth muscle layer increased in asthmatic subjects. This phenomenon was also found in the present study, where an increased density of MC_{TC} cells, but not MC_T, was found in the bronchial smooth muscle layer of uncontrolled asthmatics compared to the same compartment in healthy controls. Given that this mast cell subtype is thought to be steroid insensitive, and that several mast cell mediators have the ability to cause airway bronchoconstriction, hyperresponsiveness and remodeling, this finding supports the proposed pathophysiological effects of smooth muscle-associated mast cells in asthma[54, 55].

In this study, we found no direct correlations between mast cell parameters and lung function values within the uncontrolled asthma cohort in this study (see online supplement). However, the number of patients was small and previous studies have shown that conventional lung functional parameters might not accurately represent distal lung inflammation[56]. Indeed, it has been demonstrated that measurements of thoracic gas volume and total lung capacity better represents peripheral inflammation, and that these parameters correlate to e.g. distal eosinophilic inflammation in patients with nocturnal asthma[37].

In support of a beneficial effect of the ongoing treatment with ICS, the MC_T population decreased in central airways of asthmatics, while the MC_{TC} numbers increased. As steroids have previously been showed to reduce mast cell numbers in central airways and to mainly affect the MC_T population[57], this observation implies an effect of ICS on mast cells in the

372 bronchi. In contrast, both MC_T and MC_{TC} subpopulations increased significantly in the less
373 steroid-exposed alveolar parenchyma, which indicates that alveolar mast cell populations are
374 not well targeted by conventional ICS therapy.
375
376 Our data support the notion that patients who do not respond to conventional ICS therapy may
377 have a peripheral airway inflammation and should thus benefit from treatment strategies with
378 improved targeting of the distal lung[11, 58]. ICS, which are the foundation treatment of choice
379 for asthma patients[6, 59, 60], were originally developed to primarily treat the central airways.
380 Anti-IgE therapy (Omalizumab; Xolair®) was developed on the basis of the proposed IgE-
381 driven allergic inflammation in the conducting airways[63-65]. Omalizumab down-regulate both
382 IgE and FcεRI-bearing mast cells in asthmatic bronchi[3, 66]. Future studies are now needed to
383 investigate if anti-IgE therapy yields similar effects in the alveolar compartment.
384
385 In summary, this study has demonstrated that atopic asthma patients with persistent symptoms
386 despite conventional ICS therapy have increased MC_T and MC_{TC} populations in the alveolar
387 parenchyma. These expanded populations are characterized by markedly elevated expression
388 of FcεRI and surface-bound IgE. Apart from advancing the concept of a distal airway
389 inflammation in asthma, this observation provides important indications regarding how to
390 improve treatment strategies for uncontrolled asthma.
391

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TABLE 1. SUBJECT CHARACTERISTICS FOR UNCONTROLLED ASTHMA, AR AND HEALTHY CONTROLS

Subject	Asthma / Controls	Gender (M/F)	Age (yrs)	FEV1 (L)	FEV_1 % of pred.	PD20 (μg)	Atopy (y/n)	Rhinitis (y/n)	ICS/day (μg)	ACT score	Smoking (y/n)
1	control	M	29	5.11	116.4	>2000	n	n	0	N/A	n
2	control	F	23	3.68	95.4	>2000	n	n	0	N/A	n
3	control	M	39	4.91	109.8	>2000	n	n	0	N/A	n
4	control	M	23	5.38	113.5	>2000	n	n	0	N/A	n
5	control	F	23	3.71	100.7	>2000	n	n	0	N/A	n
6	control	F	22	2.38	72.1	>2000	n	n	0	N/A	n
7	control	F	23	3.53	95.1	>2000	n	n	0	N/A	n
8	control	F	21	3.25	89.4	>2000	n	n	0	N/A	n
9	rhinitis	M	22	3.98	108.7	>2000	y (p)	y	0[a]	N/A	n
10	rhinitis	F	25	2.98	96.3	>2000	y (p)	y	0	N/A	n
11	rhinitis	F	36	3.21	103.8	>2000	y (p)	y	0	N/A	n
12	rhinitis	M	59	3.01	138.0	>2000	y (p)	y	0[a]	N/A	n
13	rhinitis	F	24	3.42	96.0	>2000	y (p)	y	0	N/A	n
14	rhinitis	F	24	3.21	105.0	>2000	y (p)	y	0	N/A	n
15	rhinitis	M	29	4.69	114.6	>2000	y (s)	y	0	N/A	n[b]
16	rhinitis	F	31	3.68	109.3	>2000	y (p)	y	0	N/A	n
17	asthma	M	45	4.25	104.2	243.4	y (p)	y	400	13	n
18	asthma	M	59	3.17	81.0	672.0	y (p)	y	800	19	n
19	asthma	M	27	3.86	81.7	>2000	y (p)	n	800	20	n
20	asthma	M	22	3.58	79.9	251.5	y (p)	y	400	21	n
21	asthma	M	58	2.80	63.3	68.0	y (p)	y	400	13	n
22	asthma	M	30	4.01	90.1	1620.0	y (p)	y	800	18	n
23	asthma	F	50	2.14	74.7	69.3	y (p)	y	800	16	n
24	asthma	F	24	2.35	72.5	381.9	y (s)	y	1200	20	n
25	asthma	F	50	2.07	72.3	138.2	y (p)	y	800	17	n[c]
26	asthma	F	52	2.37	81.5	279.1	y (p)	y	800	14	n
27	asthma	M	38	4.64	108.0	541.0	y (p)	y	1200	17	n
28	asthma	M	37	4.14	96.0	500.0	y (p)	y	400	18	n[d]
29	asthma	M	25	3.35	73.3	59.5	y (p)	y	800	18	n
30	asthma	F	42	2.55	81.8	326.0	y (s)	y	800	11	n

M = male, F = female, FEV_1 = forced expiratory volume in 1 second, PD20 = provocative dose (metacholine) producing a fall in FEV_1 of 20 %, s = seasonal, p = perennial, ICS = Inhaled glucocorticosteroid, ACT = asthma control test, y = yes, n = no, [a] nasal corticosteroid p.r.n, [b] ex-smoker since 2003, [c] ex-smoker since 1985, [d] ex-smoker since 2001

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TABLE 2. MAST CELL DENSITIES AND EXPRESSION OF FcεRI AND MAST CELL BOUND IgE IN PATIENTS WITH UNCONTROLLED ASTHMA AND AR

		Central Airways			Alveolar Parenchyma		
Uncontrolled Asthma Density (per mm^2)		**Controls[a] (n = 8)**	**Uncontrolled asthma (n=14)**	**p-value**	**Controls (n = 8)**	**Uncontrolled asthma (n=14)**	**p-value**
	Total	69 (16-185)	40 (12-109)	*0.2*	53 (0-241)	210 (59-591)	0.006
	MC_T	60 (11-179)	19 (0-69)	0.05	33 (0-239)	178 (22-456)	0.01
	MC_{TC}	10 (0-24)	24 (5-83)	0.05	12 (0-31)	38 (5-299)	0.04
Expression (%)	FcεRI	69 (43–100)	86 (50–100)	*0.1*	3 (0–11)	81 (8–100)	0.0002
	IgE	31 (11-75)	91 (30-100)	0.003	0 (0-12)	73 (13-100)	0.0001
AR Density (per mm^2)		**Controls[b] (n=8)**	**AR (n=8)**	**p-value**	**Controls (n=8)**	**AR (n=8)**	**p-value**
	Total	79 (31-155)	83 (65-319)	*0.7*	61 (0-179)	79 (32-186)	*0.2*
	MC_T	72 (10-155)	66 (14-265)	*1.0*	46 (0-149)	64 (0-180)	*0.6*
	MC_{TC}	15 (0-28)	21 (8-61)	*0.3*	10 (0-30)	18 (6-68)	*0.2*
Expression (%)	FcεRI	73 (40-100)	76 (40-100)	*0.6*	0 (0-29)	0 (0-19)	*0.8*
	IgE	13 (0-50)	30 (0-51)	*0.2*	0 (0-17)	11 (0-37)	0.02

Data presented as median (range). [a] Paraformaldehyde fixated paraffin embedded control tissue, [b] PLP fixated cryo control tissue. n = number of patients in the group. A mean value per each patient was calculated from 2 bronchial and 2 transbronchial biopsies, respectively. The difference between the control group and disease group were then calculated using Mann-Whitney test. Result is considered significant for $p \leq 0.05$.

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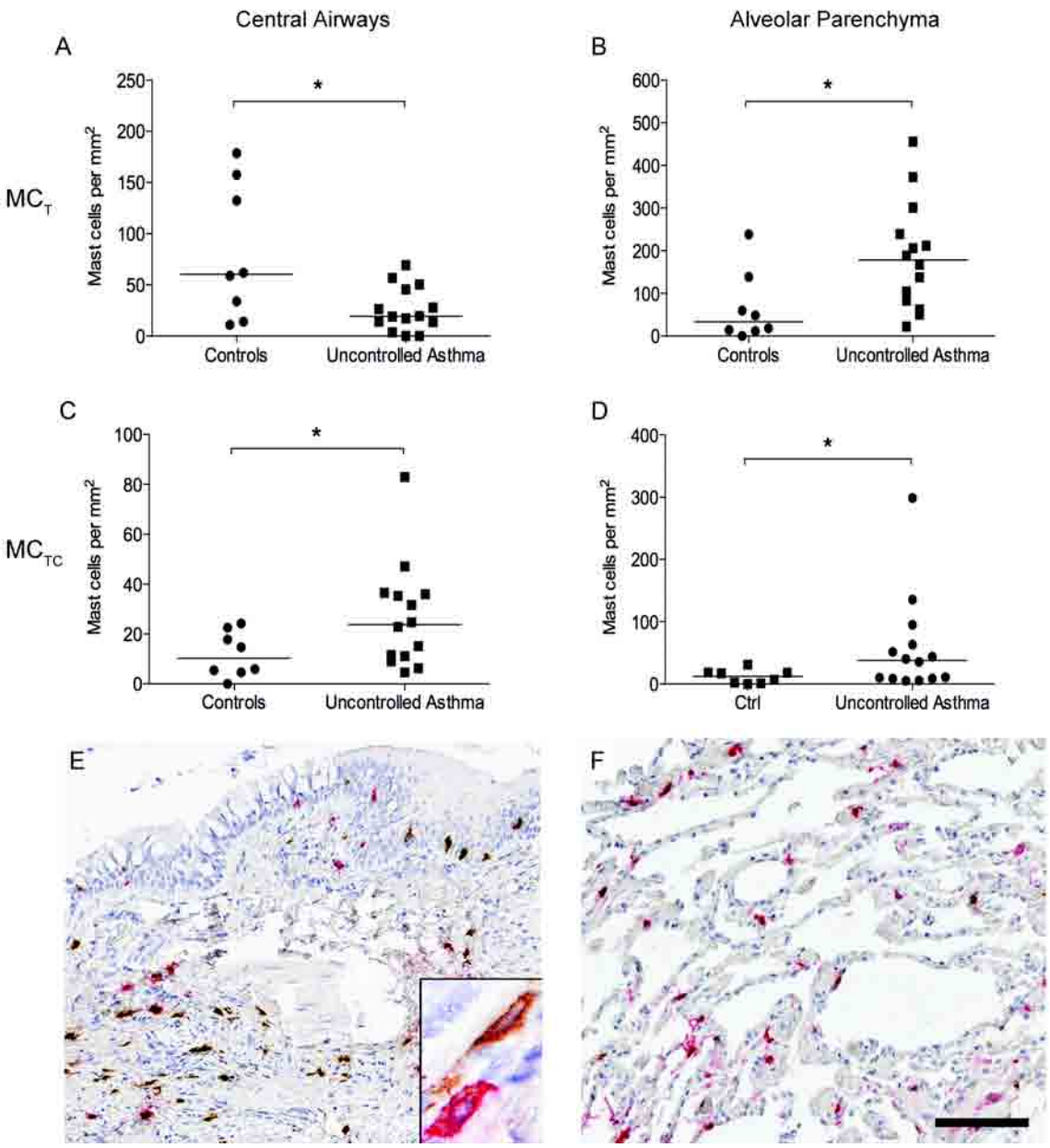
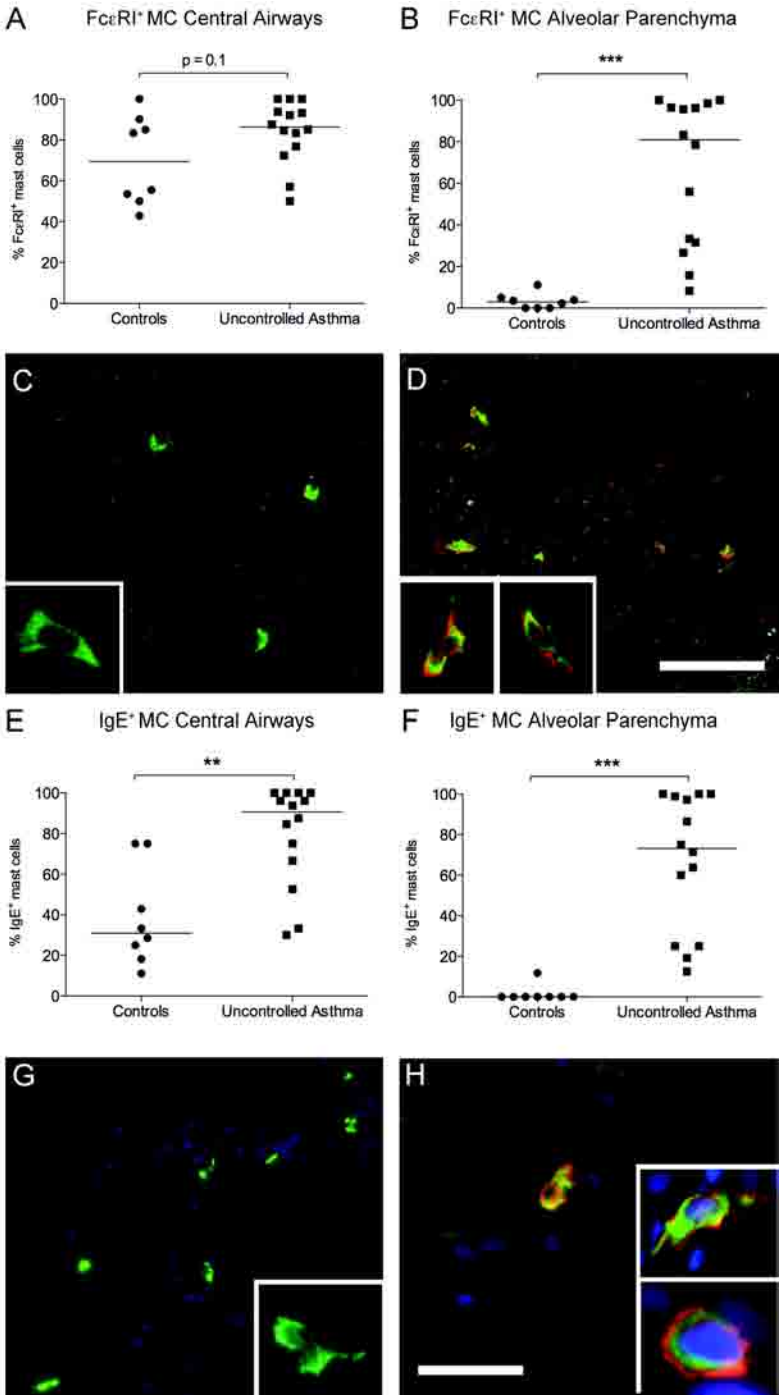


Figure 1. MC_T and MC_{TC} in central airways (A, C) and alveolar parenchyma (B, D) in uncontrolled asthma compared to healthy controls. E (central airways) and F (alveolar) show representative micrographs from asthmatic patients, double stained for MC_T and MC_{TC}. Scale bar: E-F = 100 μm. Inset in (E) represents a close-up image (600×) of neighboring MC_{TC} and MC_T cells. Horizontal bars indicates median value.



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580 **Figure 2.** Mast cell expression (%) of FcεRI (A-B), panel C-D show representative
581 micrographs of FcεRI$^+$ mast cells in alveolar parenchyma from controls (C) and asthmatic
582 patients (D). E-F show mast cell bound IgE (%), panel G-H show representative micrographs
583 of IgE$^+$ mast cells in alveolar parenchyma from controls (G) and asthmatic patients (H). Scale
584 bar: C-D, G = 50 μm and H = 25 μm. Insets represents image (600×) of mast cells double
585 positive for tryptase and FcεRIα or IgE. Horizontal bars indicates median value.

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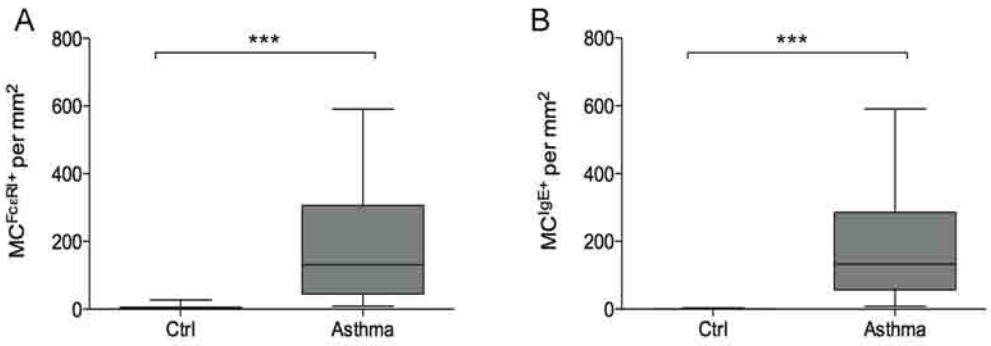

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588 **Figure 3.** Density of mast cells expressing FcεRIα (A) and mast cell bound IgE (B) in
589 alveolar parenchyma in uncontrolled asthma compared to healthy controls. Data are presented
590 as box and whiskers.