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

Andersson, Cecilia; Bergqvist, Anders; Mori, Michiko; Mauad, Thais; Bjermer, Leif; Erjefält, Jonas

Published in:
Journal of Allergy and Clinical Immunology

DOI:
10.1016/j.jaci.2011.01.022

2011

Link to publication

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
MAST CELL-ASSOCIATED ALVEOLAR INFLAMMATION IN PATIENTS WITH ATOPIC UNCONTROLLED ASTHMA.

Cecilia K Andersson, MSc¹, Anders Bergqvist¹, MSc¹, Michiko Mori, MSc², Thais Mauad, MD, PhD³, Leif Bjermer, MD, PhD¹, and Jonas S Erjefält, PhD¹,²

¹Department of Respiratory Medicine and Allergology, Lund University, Lund, Sweden
²Department of Experimental Medical Science, Lund University, Lund, Sweden
³Department of Pathology, São Paulo University, São Paulo, Brazil

Corresponding author and requests for reprints should be addressed to:
Jonas Erjefält, Assoc. Prof., e-mail: jonas.erjefalt@med.lu.se
Airway Inflammation Unit, Dept. of Experimental Medical Science,
BMC D12, Lund University, SE-22184, Lund, Sweden
Phone: +46 46 222 0960, Fax: +46 46 211 3417


Word count: 3256
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≤0.05), while MC_TC increased (p≤0.05). In the alveolar parenchyma from asthmatics an increased density was found for both MC_T (p≤0.05) and MC_TC (p≤0.05). 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 (FceRI).

Key words: mast cells; asthma; FceRI; 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\textsubscript{TC}: tryptase and chymase positive mast cells (connective tissue mast cells)

MC\textsubscript{T}: tryptase positive mast cells (mucosal mast cells)

HRP: horseradish peroxidase

DAB: 3,3’ diaminobenzidine

AP: alkaline peroxidase

PD\textsubscript{20}: cumulative dose of bronchoconstrictor where FEV\textsubscript{1} fell by 20 % or more

FEV\textsubscript{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, Fc\(\varepsilon\)RI\(^3, 4\). 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 Fc\(\varepsilon\)RI\(^15\). 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 Fc\(\varepsilon\)RI 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\textsuperscript{10, 12, 17, 21, 22}. From this, and the fact that high IgE-levels may lead to increased FceRI expression on mast cells\textsuperscript{23, 24}, we hypothesized that patients with ICS-treated, atopic uncontrolled asthma have a significantly altered mast cell population where the normally FceRI low-expressing alveolar mast cells have acquired an FceRI-expressing phenotype. To test this hypothesis mast cell densities and mast cell expression of FceRI 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)\(^6\). 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).
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.

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.

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 (FceRI) or surface-bound IgE (see online supplement and Table E1).

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 / mm² tissue) was calculated for each mast cell subtype\textsuperscript{14, 19, 20, 30, 35-37}.

**Quantification of FceRI\textsuperscript{+} and IgE\textsuperscript{+} 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\textsuperscript{FceRI\textsuperscript{+}} or MC\textsuperscript{IgE\textsuperscript{+}} with the total mast cell density in the same tissue region (for further details and quantification on COPD and CF, see ref \textsuperscript{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 ≤ 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\textsubscript{1} % predicted was lower in patients with asthma (81 [63-108] FEV\textsubscript{1} % pred.) compared to healthy controls (98 [72-116] FEV\textsubscript{1} % pred., p = 0.03) and patients with rhinitis (107 [96-138] FEV\textsubscript{1} % pred., p = 0.001). No difference in FEV\textsubscript{1} % pred. was found between controls and rhinitis (p = 0.3). In addition, 5 CF and 10 COPD patients were investigated\textsuperscript{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
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\epsilon RIα^+}/mm^2) compared to controls (1.5 [0-27] MC^{Fc\epsilon RIα^+}/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
In AR, no significant change in total mast cell numbers or the density of MC_1 and MC_{TC} was found in central airways or in alveolar parenchyma compared to healthy controls (Table 2). No increase in the proportion of mast cells expressing the FcεRIα could be found in central airways or in the alveolar parenchyma in patients with AR compared to controls (Table 2). No significant increase in the proportion of mast cells with surface bound IgE was found in central airways. However, an increase in the proportion of mast cells with surface bound IgE was found in alveolar parenchyma in patients with AR compared to controls (Table 2).

Non-Allergic Lung Diseases: Comparison to COPD and CF

The FcεRIα expression was high in central airways in controls compared to the same compartment in COPD and CF. In alveolar parenchyma, the mast cell FcεRIα expression was low in controls; no significant change was found in COPD and CF patients (see online supplement).
DISCUSSION

Accumulated evidence from physiological studies and tissue explorations suggest that inflammatory processes in the distal airways contribute to asthma pathogenesis. 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 FceRI-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 FceRI\(^15\). The alveolar mast cells, however, have under healthy conditions a very low FceRI expression\(^19\). In this study the number of FceRI 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. The vast majority of patients with asthma have rhinitis and rhinitis, especially at severe stages, is a major risk factor for developing asthma. 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 IgE R.

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. 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. 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 asthmatics27.

In 2002, Brightling et al17 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\textsubscript{TC} cells, but not MC\textsubscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{37}.

In support of a beneficial effect of the ongoing treatment with ICS, the MC\textsubscript{T} population decreased in central airways of asthmatics, while the MC\textsubscript{TC} numbers increased. As steroids have previously been showed to reduce mast cell numbers in central airways and to mainly affect the MC\textsubscript{T} population\textsuperscript{57}, this observation implies an effect of ICS on mast cells in the
bronchi. In contrast, both \( M_{CT} \) and \( M_{CTC} \) subpopulations increased significantly in the less steroid-exposed alveolar parenchyma, which indicates that alveolar mast cell populations are not well targeted by conventional ICS therapy.

Our data support the notion that patients who do not respond to conventional ICS therapy may have a peripheral airway inflammation and should thus benefit from treatment strategies with improved targeting of the distal lung\(^{11,58} \). ICS, which are the foundation treatment of choice for asthma patients\(^{6,59,60} \), were originally developed to primarily treat the central airways. Anti-IgE therapy (Omalizumab; Xolair®) was developed on the basis of the proposed IgE-driven allergic inflammation in the conducting airways\(^{63-65} \). Omalizumab down-regulate both IgE and FcεRI-bearing mast cells in asthmatic bronchi\(^3,66 \). Future studies are now needed to investigate if anti-IgE therapy yields similar effects in the alveolar compartment.

In summary, this study has demonstrated that atopic asthma patients with persistent symptoms despite conventional ICS therapy have increased \( M_{CT} \) and \( M_{CTC} \) populations in the alveolar parenchyma. These expanded populations are characterized by markedly elevated expression of FcεRI and surface-bound IgE. Apart from advancing the concept of a distal airway inflammation in asthma, this observation provides important indications regarding how to improve treatment strategies for uncontrolled asthma.
ACKNOWLEDGEMENTS

We thank Karin Jansner and Britt-Marie Nilsson for skillful technical assistance with tissue processing and immunohistochemical staining.
REFERENCES


30. KleinJan A, McEuen AR, Dijkstra MD, Buckley MG, Walls AF, Fokkens WJ. Basophil and eosinophil accumulation and mast cell degranulation in the nasal mucosa of


497  40. Csaba G, Kovacs P, Pallinger E. Gender differences in the histamine and
498  serotonin content of blood, peritoneal and thymic cells: a comparison with mast cells. Cell
500  41. Dunlop SP, Jenkins D, Spiller RC. Age-related decline in rectal mucosal
502  42. Greisner WA, 3rd, Settipane RJ, Settipane GA. Co-existence of asthma and
505  43. Guerra S, Sherrill DL, Martinez FD, Barbee RA. Rhinitis as an independent risk
507  44. Linneberg A, Henrik Nielsen N, Frolund L, Madsen F, Dirksen A, Jorgensen T.
508  The link between allergic rhinitis and allergic asthma: a prospective population-based study.
509  The Copenhagen Allergy Study. Allergy 2002; 57:1048-52.
510  45. Settipane RJ, Hagy GW, Settipane GA. Long-term risk factors for developing
511  asthma and allergic rhinitis: a 23-year follow-up study of college students. Allergy Proc 1994;
512  15:21-5.
514  Dust mite allergens are carried on not only large particles. Pediatr Allergy Immunol 1999;
515  10:258-60.
516  47. Taylor PE, Flagan RC, Miguel AG, Valenta R, Glovsky MM. Birch pollen
518  48. Taylor PE, Flagan RC, Valenta R, Glovsky MM. Release of allergens as
519  respirable aerosols: A link between grass pollen and asthma. J Allergy Clin Immunol 2002;


545 58. Hamid Q, Tulic MK. New insights into the pathophysiology of the small
547 59. FitzGerald JM, Shahidi N. Achieving asthma control in patients with moderate
551 response to omalizumab, an anti-IgE antibody, in patients with allergic asthma. Chest 2004;
552 125:1378-86.
553 62. Jabara HH, Ahern DJ, Vercelli D, Geha RS. Hydrocortisone and IL-4 induce
555 63. Holgate S, Smith N, Massanari M, Jimenez P. Effects of omalizumab on
556 markers of inflammation in patients with allergic asthma. Allergy 2009; 64:1728-36.
557 64. Busse WW. Anti-immunoglobulin E (omalizumab) therapy in allergic asthma.
560 354:2689-95.
562 effect of treatment with omalizumab, an anti-IgE antibody, on asthma exacerbations and
564
565
566
### TABLE 1. SUBJECT CHARACTERISTICS FOR UNCONTROLLED ASTHMA, AR AND HEALTHY CONTROLS

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

M = male, F = female, FEV1 = forced expiratory volume in 1 second, PD20 = provocative dose (metacholine) producing a fall in FEV1 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
<table>
<thead>
<tr>
<th></th>
<th>Central Airways</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncontrolled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Density (per mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>69 (16-185)</td>
<td>40 (12-109)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>MCT</td>
<td>60 (11-179)</td>
<td>19 (0-69)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>MCTC</td>
<td>10 (0-24)</td>
<td>24 (5-83)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Expression (%)</td>
<td>FcεRI</td>
<td>69 (43–100)</td>
<td>86 (50–100)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>IgE</td>
<td>31 (11-75)</td>
<td>91 (30-100)</td>
<td>0.003</td>
</tr>
<tr>
<td>AR Density (per mm²)</td>
<td>Controls</td>
<td>AR (n=8)</td>
<td>p-value</td>
<td>Controls</td>
</tr>
<tr>
<td>Total</td>
<td>79 (31-155)</td>
<td>83 (65-319)</td>
<td>0.7</td>
<td>61 (0-179)</td>
</tr>
<tr>
<td>MCT</td>
<td>72 (10-155)</td>
<td>66 (14-265)</td>
<td>1.0</td>
<td>46 (0-149)</td>
</tr>
<tr>
<td>MCTC</td>
<td>15 (0-28)</td>
<td>21 (8-61)</td>
<td>0.3</td>
<td>10 (0-30)</td>
</tr>
<tr>
<td>Expression (%)</td>
<td>FcεRI</td>
<td>73 (40-100)</td>
<td>76 (40-100)</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>IgE</td>
<td>13 (0-50)</td>
<td>30 (0-51)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data presented as median (range). *Paraformaldehyde fixed paraffin embedded control tissue, b PLP fixed 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 ≤ 0.05.
Figure 1. MC<T> and MC<T>C 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<T>C. Scale bar: E-F = 100 µm. Inset in (E) represents a close-up image (600×) of neighboring MC<T>C and MC<T> cells. Horizontal bars indicates median value.
Figure 2. Mast cell expression (%) of FcεRI (A-B), panel C-D show representative micrographs of FcεRI⁺ mast cells in alveolar parenchyma from controls (C) and asthmatic patients (D). E-F show mast cell bound IgE (%), panel G-H show representative micrographs of IgE⁺ mast cells in alveolar parenchyma from controls (G) and asthmatic patients (H). Scale bar: C-D, G = 50 μm and H = 25 μm. Insets represents image (600×) of mast cells double positive for tryptase and FcεRIα or IgE. Horizontal bars indicates median value.
Figure 3. Density of mast cells expressing FcεRIα (A) and mast cell bound IgE (B) in alveolar parenchyma in uncontrolled asthma compared to healthy controls. Data are presented as box and whiskers.