

# Relationship of Small Airway Chymase-Positive Mast Cells and Lung Function in Severe Asthma

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Distal lung inflammation may be important in asthma pathophysiology. The goal of this study was to measure cellular inflammation in the large airway and four distal lung regions (small airway inner and outer wall, alveolar attachments, and peripheral alveolar tissue) and to correlate the specific inflammatory cells with several lung function parameters. Sections of concurrently obtained endobronchial and transbronchial/surgical biopsy tissue from 20 individuals with severe asthma were immunostained for T-lymphocyte, eosinophil, monocyte/macrophage, neutrophil, and two mast cell markers (tryptase and chymase). Specific cell distributions were determined and correlated with lung function measures. The number of inflammatory cells generally increased toward the periphery, but the percentage of T-lymphocytes, eosinophils, monocytes/macrophages, and neutrophils remained similar or decreased from large to small airways. In contrast, mast cell number, percentage, and the chymase-positive phenotype increased in small airway regions. After the analysis was adjusted for multiple comparisons, only chymase-positive mast cells significantly and positively correlated with lung function. Such a relationship was seen only in the small airway/alveolar attachments lung region ( $r_s = 0.61$ – $0.89$ ;  $p \leq 0.001$  for all correlations). These data suggest that induction of chymase-positive mast cells, particularly in the small airway outer wall/alveolar attachments region, may be protective for lung function in severe asthma.

**Keywords:** bronchial asthma; inflammation; mast cells

Airway inflammation likely drives a considerable portion of the pathophysiologic changes of asthma. Although the majority of studies have analyzed the inflammatory process in large airways, physiologic, radiologic, and pathologic evidence support the presence of an inflammatory process in the distal lung regions as well (1, 2).

Despite the concept that changes in the lung periphery could drive physiologic and clinical abnormalities associated with asthma, such studies are difficult, and only a small number of studies, using tissue obtained at the time of tumor resection or alveolar tissue (only) from transbronchial biopsies (TBBX) have evaluated distal lung inflammatory cell distribution (3–10). Therefore, knowledge about those distal lung regions in living subjects with asthma (or, for that matter, in normal subjects) is scarce and data on the relationship between cellular inflammation in lung periphery and lung function are minimal. Only occasional studies have attempted correlations between lung function and alveolar inflammation, but none have evaluated the relationship with airway inflammation (9, 10).

It is possible to assess inflammation in distal lung regions, including small airways of patients with asthma using a recently published methodology to identify small airway tissue in TBBX (11). Therefore, the objective of this study was to evaluate the cellular inflammation (as defined by numbers of T-lymphocytes, monocytes/macrophages, neutrophils, eosinophils, and two mast cell phenotypes) present in proximal airways and four distal lung regions of subjects with severe asthma and to correlate it with different measurements of lung function. It was hypothesized that the type, amount, and specific location of distal lung inflammation could affect lung function in asthma.

Results of this study suggest that, of all inflammatory cells analyzed, only mast cells with increased chymase expression, positioned within the small airway outer wall/alveolar attachments region, correlated (positively) with lung function of subjects with severe asthma.

Some of the results of these studies have been previously reported in abstract form (12).

## METHODS

### Tissue Acquisition

Tissue samples from proximal and distal lung regions of 20 subjects with severe, steroid-dependent asthma were obtained by concurrent endobronchial biopsy and TBBX or surgical biopsy performed for clinical reasons (*see* details in online supplement) (8, 13). This study was approved by the Institutional Review Board of the National Jewish Medical and Research Center. All subjects gave informed consent. Normal lung tissue samples ( $n = 3$ ) were obtained through National Disease Research Interchange, treated and shipped as transplant tissue and processed per protocol.

### Tissue Processing/Immunostaining

Sections of acetone-fixed/glycol-methacrylate-embedded tissue were immunostained for markers of T-lymphocytes, monocytes/macrophages, neutrophils, eosinophils, and mast cells (tryptase and chymase) (*see* online supplement).

### Definition of the Lung Regions and Morphometric Analysis

*Large airways* were analyzed in endobronchial biopsy tissue samples in the area between the subepithelial basement membrane and the smooth muscle layer (large airway inner wall).

*Small airway* in TBBX was defined as airway tissue without cartilage or mucous glands and having alveolar tissue attached to it (11). A smooth muscle layer was observed in all TBBX with small airway tissue. Two small airway regions were analyzed separately (Figure 1): *small airway inner wall* ( $SA_{iw}$ ), the area from the subepithelial basement membrane to the inner edge of the smooth muscle layer, and *small airway outer wall* ( $SA_{ow}$ ), the area from the outer edge of the smooth muscle layer to the alveolar attachments. Small airway in the open lung biopsy tissue samples was defined as an airway with a subepithelial basement membrane perimeter of less than 6 mm, without cartilage or mucous glands.

*Alveolar attachments* region was the region of alveolar tissue structurally connected to small airways. It was analyzed in TBBX that contained small airway tissue and in surgical tissue samples, within the alveolar tissue area visible at 100 $\times$  magnification (Figure 1; *see* online supplement).

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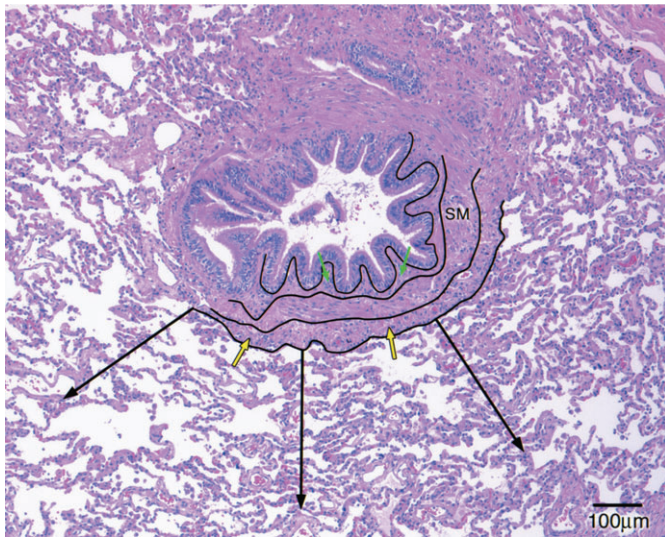


**Figure 1.** Outline of distal lung regions in hematoxylin/eosin-stained lung tissue. Small airway inner wall (green arrows), small airway outer wall (yellow arrows), and alveolar attachments region (alveolar tissue spanning from the attachments to small airway wall and toward periphery up to two visual fields at 400 $\times$  magnification). SM = smooth muscle.

Peripheral alveolar tissue was analyzed in TBBX that contained alveolar tissue only and therefore was considered to be peripheral from the alveolar attachments region.

Morphometric analysis was performed as described in the online supplement, with cell counts normalized per tissue area (per square millimeter). The total inflammatory cell population consisted of T-lymphocytes, monocytes/macrophages, neutrophils, eosinophils, and mast cells (tryptase-positive, MC<sub>T</sub>). In addition to tryptase, consecutive tissue sections were stained for chymase, a second mast cell-specific protease. A chymase-positive mast cell (MC<sub>TC</sub>)/MC<sub>T</sub> ratio was calculated to determine the percentage of the MC<sub>T</sub> population that expresses chymase.

Lung function parameters measured were: forced expiratory volume in 1 second (FEV<sub>1</sub>), forced vital capacity (FVC), FEV<sub>1</sub>/FVC, FVC/SVC (slow vital capacity), residual volume (RV), and forced expiratory flow at 25–75% of FVC (FEF<sub>25–75%</sub>) (see also online supplement).

### Statistical Analysis

Inflammatory cell counts/percentages were transformed to the natural log scale and compared among the five lung regions using repeated measures analyses. When an overall significant difference was found between regions for a particular variable, interregion comparisons were made using the Tukey-Kramer procedure.

Spearman's correlations between lung function variables and inflammatory cell counts/percentages were determined for each lung region separately. The false discovery rate procedure was applied to control for false findings due to the large number of correlations performed (14) (see online supplement).

## RESULTS

### Subject Characteristics

Twenty subjects with severe asthma (10 women, mean  $\pm$  SD age of 36  $\pm$  9 years) met criteria for severe disease as previously reported (13). They had an FEV<sub>1</sub> of 49  $\pm$  17% on 23  $\pm$  16 mg/day of prednisone. None were current smokers (five subjects were former smokers with two to seven pack-years) and none had other diseases unrelated to asthma (see online supplement).

Normal peripheral lung tissue was from a 50- and a 78-year-old woman and a 64-year-old man, all healthy nonsmokers and potential organ donors who died after an acute event.

### Morphometric Analysis

Cellular inflammation in severe asthma was evaluated in concurrently obtained samples from proximal and distal lung. Large airway tissue was available from 19 of 20 subjects. For the distal lung regions, 12 subjects had small airway with alveolar attachments tissue, 10 of these 12 had additional tissue samples with peripheral alveolar tissue only, whereas the remaining 8 subjects had tissue samples with peripheral alveolar tissue only.

The range of tissue area analyzed for large airway data was 0.15–4.1 mm<sup>2</sup>, 0.01–0.17 mm<sup>2</sup> for small airway inner wall, 0.03–0.46 mm<sup>2</sup> for small airway outer wall, 0.03–0.82 mm<sup>2</sup> for alveolar attachments, and 0.15–0.73 mm<sup>2</sup> for peripheral alveolar tissue.

### Comparison of Inflammatory Cell Numbers and Percentages in Lung Regions of Subjects with Severe Asthma

The total inflammatory cell numbers (sum of the individual cell types) were higher in SA<sub>iw</sub> (663/mm<sup>2</sup> [522–842]), SA<sub>ow</sub> (656/mm<sup>2</sup> [504–853]), and alveolar attachments (582/mm<sup>2</sup> [508–668]) than in large airway (219/mm<sup>2</sup> [188–255]; overall  $p = 0.0016$ ;  $p = 0.006$ ,  $p = 0.005$ , and  $p = 0.001$  for intergroup comparisons, respectively, but not significantly different between large airway and peripheral alveolar tissue (397/mm<sup>2</sup> [348–453];  $p = 0.11$ ).

T-lymphocyte numbers (Figure 2A) and percentages (see Figure 3 for all percentages) were higher in airways regions than in either alveolar tissue region. The percentage of T-lymphocytes in the large airway was higher than in alveolar attachments ( $p = 0.03$ ) and peripheral alveolar tissue ( $p = 0.05$ ), and also higher in SA<sub>ow</sub> than in alveolar attachments ( $p = 0.03$ ).

Eosinophil numbers (Figure 2B) and percentages were not significantly different between the lung regions.

Monocyte/macrophage numbers were increased in the periphery (Figure 2C), but the percentages were not significantly different across the lung regions.

Neutrophils were differentially distributed in airways as compared with alveolar tissue regions: the numbers in large airway and SA<sub>ow</sub> were lower than in both alveolar tissue regions, where the increase was likely from neutrophils inside capillaries (Figure 2D). Additionally, the percentage of neutrophils in the SA<sub>ow</sub> was the lowest among all regions (3% [2–5]) and significantly less than in large airway, alveolar attachments, and peripheral alveolar tissue ( $p < 0.0001$  for all three regions).

Mast cell numbers (MC<sub>T</sub>, Figure 2E) were increased in the lung periphery as compared with the large airway, particularly in the SA<sub>ow</sub> and alveolar attachments. The number of mast cells in the SA<sub>ow</sub> was higher than in all other lung regions except SA<sub>iw</sub>. Finally, the mast cell percentage in the SA<sub>ow</sub> was higher than in any other lung region: SA<sub>iw</sub> ( $p = 0.04$ ), alveolar attachments ( $p = 0.002$ ), peripheral alveolar tissue ( $p = 0.0001$ ), and large airway ( $p = 0.007$ ), and was the predominant inflammatory cell present in this region.

### Distribution of Chymase-positive Mast Cells (MC<sub>TC</sub>) and MC<sub>TC</sub>/MC<sub>T</sub> Ratio in Lung Regions of Subjects with Severe Asthma

Because the mast cell was the prominent cell in the lung periphery, colocalization studies were performed and the percentage of the MC<sub>T</sub> that were also chymase-positive (MC<sub>TC</sub>) was determined (Figure 4; examples of tryptase and chymase staining in the small airway/alveolar attachments can be seen in Figure 5). The numbers of MC<sub>TC</sub> and the MC<sub>TC</sub>/MC<sub>T</sub> ratio were significantly different between the lung regions (overall  $p = 0.0008$  and  $p = 0.001$ , respectively). MC<sub>TC</sub> were present in higher numbers in SA<sub>iw</sub> and alveolar attachments than in large airway ( $p = 0.008$  and  $p = 0.007$ , respectively) and peripheral alveolar tissue ( $p = 0.01$  for both comparisons). Similarly, the MC<sub>TC</sub>/MC<sub>T</sub> ratios in both the SA<sub>iw</sub> (41% [27–62]) and alveolar attachments (52%

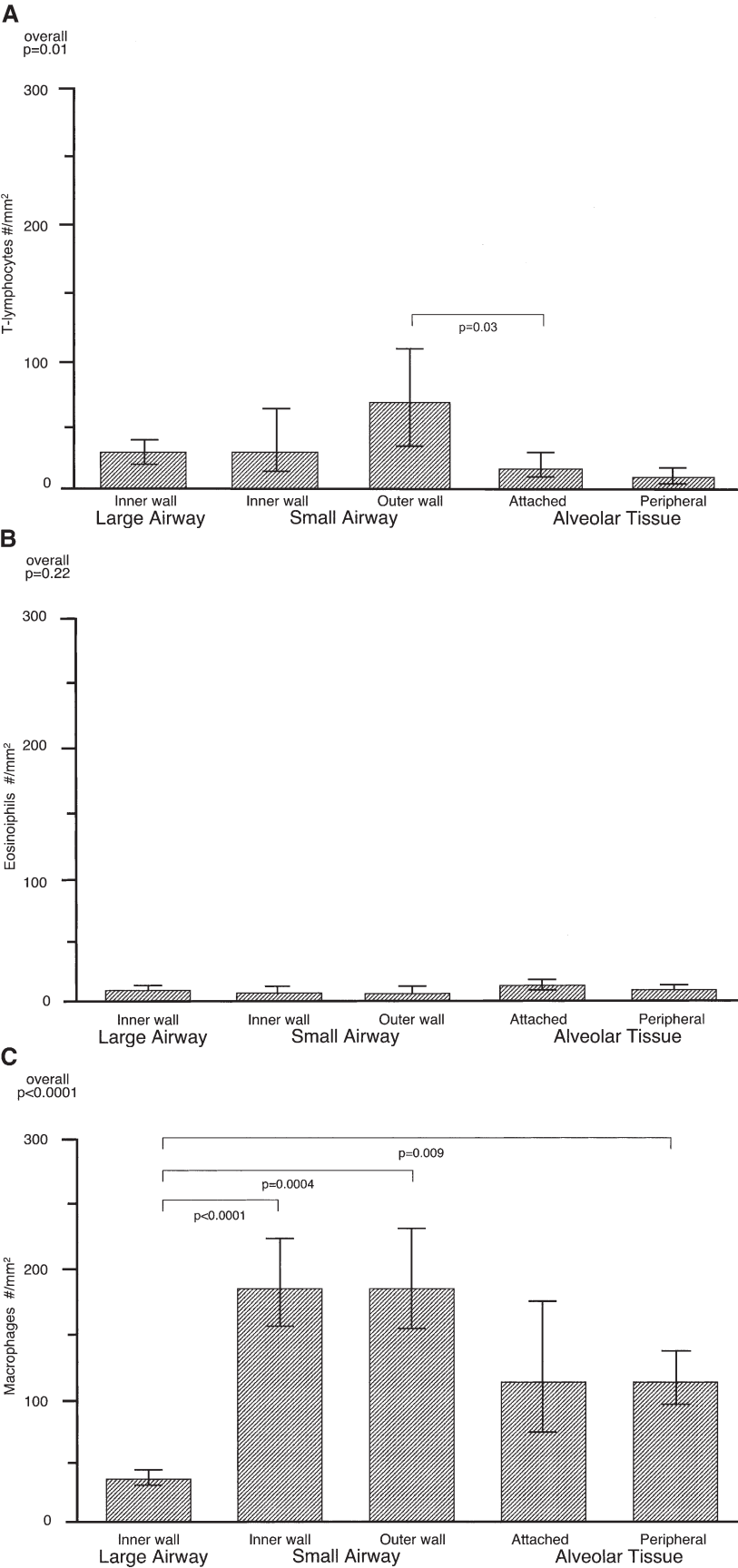


**Figure 2.** Distribution of lymphocytes (A), eosinophils (B), monocytes/macrophages (C), neutrophils (D), and mast cells (E) in five lung regions of subjects with severe asthma. Each column represents estimates of mean values  $\pm$  SE, as described in STATISTICAL ANALYSIS.

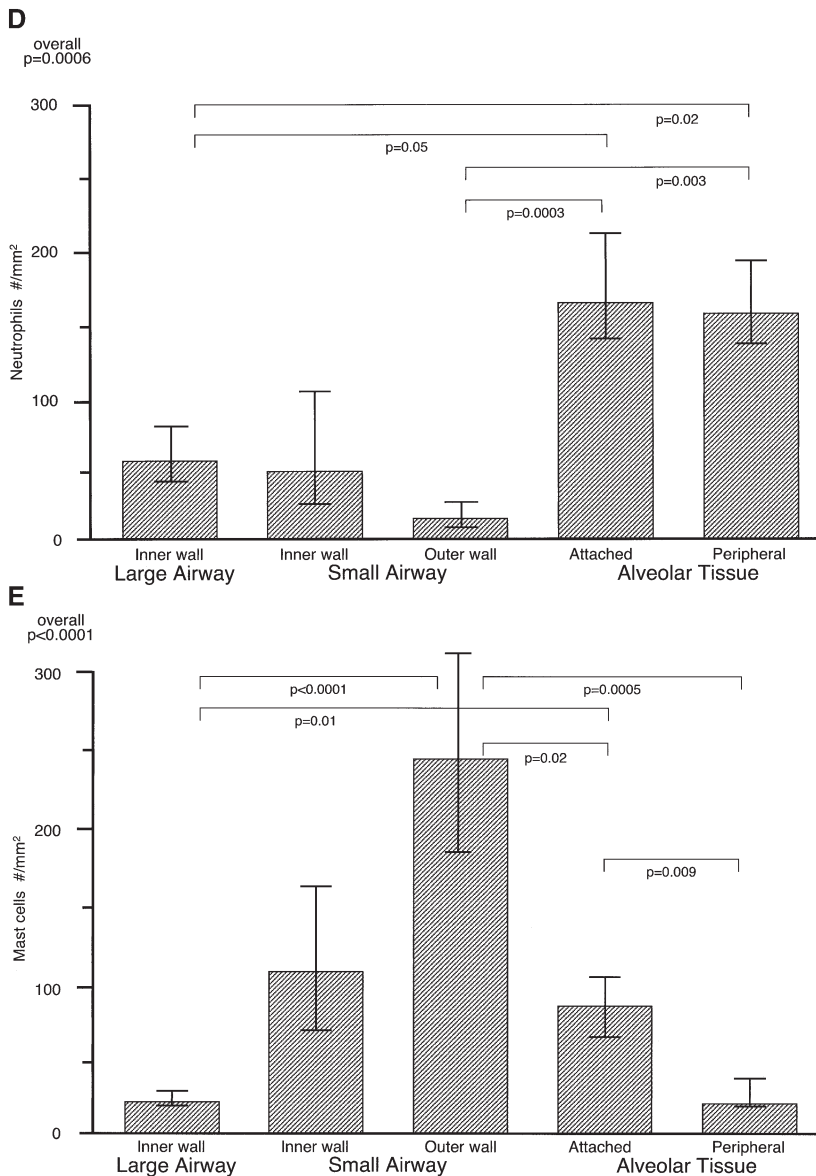


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[44–63]) were higher than in peripheral alveolar tissue (6% [3–9];  $p = 0.03$  and  $p = 0.005$ , respectively). In 3 of 12 subjects, not all  $MC_{TC}$  could be colocalized with tryptase-positive cells (i.e., there were greater numbers of  $MC_{TC}$  than  $MC_T$ ). Consequently, the  $MC_{TC}/MC_T$  ratios were above 100% (range 107–301%) in those subjects (Figure 6G). Although an occasional gain or loss of positive cells between two consecutive sections may offer some explanation, in one subject there were greater numbers of  $MC_{TC}$  in all lung regions except peripheral alveolar tissue.

#### Relationship between Inflammatory Cell Numbers/Percentages and Lung Function in Severe Asthma

Lung function values for the 20 subjects with severe asthma (mean  $\pm$  SEM) were:  $FEV_1 = 50 \pm 4\%$  predicted,  $FVC = 73 \pm 4\%$  predicted,  $FEV_1/FVC = 57 \pm 3\%$ ,  $FVC/\text{slow vital capacity} = 90 \pm 2\%$ ,  $RV = 189 \pm 10\%$  predicted and  $FEF_{25-75\%} = 28 \pm 4\%$  predicted.

Correlations ( $p < 0.05$  [unadjusted]) of total inflammatory cell counts and specific cell counts/percentages with six lung function parameters in each of the five lung regions are presented in Table 1A. All regions except peripheral alveolar tissue had

from one to four such correlations. The eosinophil numbers and percentages in the large airway consistently correlated with worsening of lung function. However, none of the correlations between the basic inflammatory cell type counts/percentages and lung function measures were significant after applying the false discovery rate procedure.

Table 1B presents the correlations with  $p < 0.05$  (unadjusted) for the subset of mast cells that express chymase. Consistent, significant, and positive relationships existed between lung function and the number of  $MC_{TC}$  as well as the  $MC_{TC}/MC_T$  ratio in the  $SA_{ow}$  and alveolar attachments. Of all the correlations evaluated, only these maintained significance after performing the false discovery rate procedure.

#### Comparison of Inflammatory Cell Distribution in Small Airway Regions of Subjects with Severe Asthma to Normal Subjects

To begin to address similarities and differences in inflammatory cell distribution in distal lung regions of subjects with severe asthma as compared with normal controls, distal lung tissue was obtained from three healthy organ donors who died suddenly

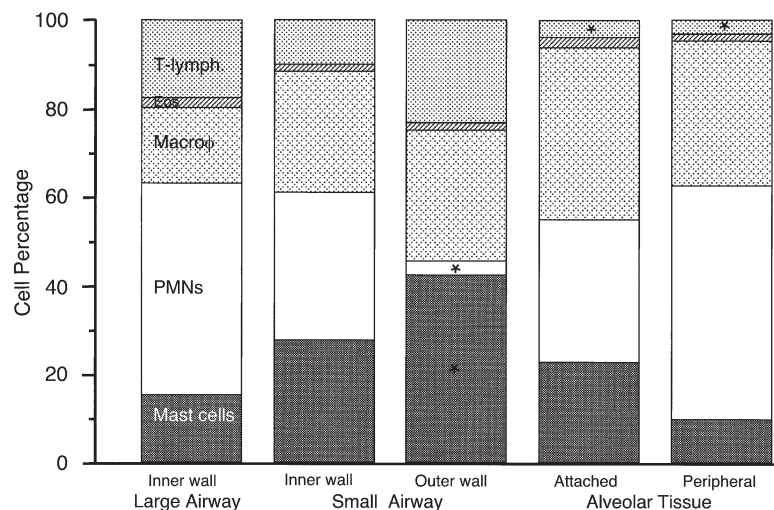


**Figure 3.** Percentages of specific inflammatory cell types in the five lung regions of subjects with severe asthma. Each column consists of the mean percentage values for each cell type, which have been proportionally adjusted to 100%. Macroph = monocytes/macrophages; PMNs = neutrophils. \* $p < 0.05$  for comparison with same variable in large airway.

and whose lungs were, for various reasons, unsuitable for transplantation. The small sample size limits statistical evaluation. Nevertheless, there were no large differences between T-lymphocytes, macrophages, neutrophils, eosinophils, and mast cells numbers and percentages in distal lung regions of normal subjects (Figures 6A–6E). However, as shown in Figures 6F and 6G, although the number of total mast cells in SA<sub>iw</sub>, SA<sub>ow</sub>, and alveolar attachments appeared similar, the number of MC<sub>TC</sub> and the MC<sub>TC</sub>/MC<sub>T</sub> ratio were higher in those with severe asthma when compared to normal subjects ( $p < 0.01$  for both number and percentage of MC<sub>TC</sub> in alveolar attachments, even with the very small sample size).

## DISCUSSION

This study suggests that significant differences exist in the amount and composition of cellular infiltrate between proximal and distal lung regions analyzed from subjects with severe asthma, particularly regarding mast cell distribution and phenotypes. Although several previous studies have evaluated inflammatory cell counts in small airways from living asthmatics in resected lung tissue or in alveolar tissue from TBBX, this is the first comprehensive analysis of both airway and parenchymal inflammation to correlate lung function with inflammation in

small airways and alveolar attachments (3–10). Unexpectedly, the strongest relationship with lung function was found for chymase-positive mast cells in the region structurally connecting small airway with lung parenchyma (i.e., small airway outer wall and alveolar attachments region, where an increased number of chymase-positive mast cells correlated with better lung function).

To begin to characterize the cellular inflammation throughout the lung in subjects with severe asthma, evaluation of multiple types of inflammatory cells was undertaken. Comparison between the corresponding proximal large airway inner wall and distal small airway inner wall regions revealed no qualitative/percentage differences, but consistent with previous studies, the cellular infiltrate increased in density from proximal to distal lung (11). Similar to previous autopsy studies, eosinophils and T-lymphocytes were evenly distributed throughout the airways of subjects with severe asthma (15). The eosinophils in the large airways correlated with worsening of lung function as previously reported by others (13, 16–18). Monocytes/macrophages increased from large airway to small airway, without change in percentages or consistent relationship to lung function. Neutrophil percentages decreased in the periphery and were lowest in the SA<sub>ow</sub>. No correlations between neutrophils in any of the lung regions and lung function were detected.

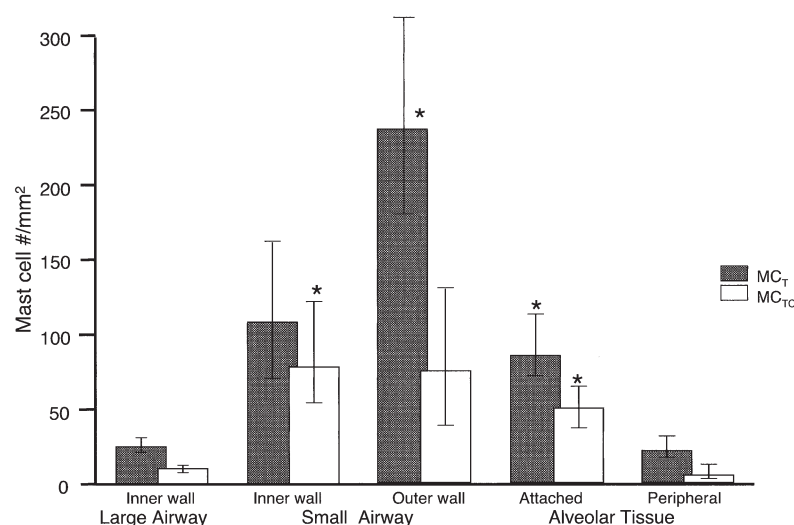


**Figure 4.** Subset of chymase-expressing mast cells (white bars) (MC<sub>T</sub>) within the total mast cell populations (bars with pattern) (MC<sub>TC</sub>) in the five lung regions. Each column represents estimates of mean values  $\pm$  SE, as described in STATISTICAL ANALYSIS. \* $p < 0.05$  for comparison with same variable (MC<sub>T</sub> or MC<sub>TC</sub>) in large airway.

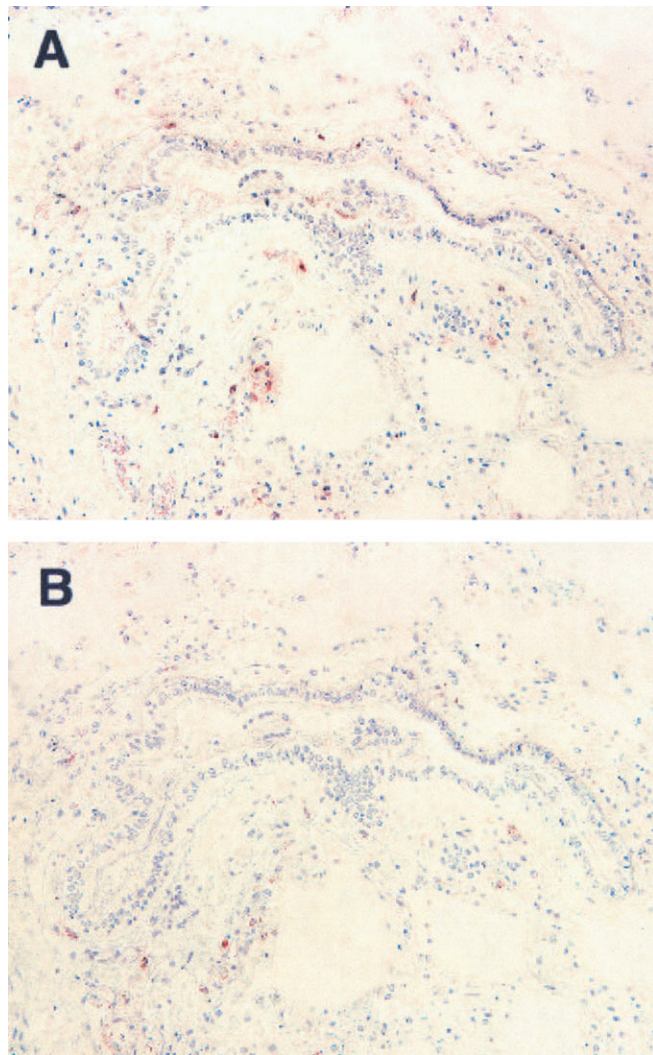


**Figure 5.** Tryptase-positive (A) and chymase-positive (B) mast cells in consecutive tissue sections of transbronchial biopsy containing small airway tissue (immunohistochemical staining, 100 $\times$ ).

Mast cells number and percentages in severe asthmatics markedly increased toward the periphery, peaking in the small airway outer wall. Surprisingly, total mast cell counts did not significantly correlate with lung function in any of the lung regions. However, a subset of mast cells expressing chymase was larger in the small airway/alveolar attachments region in subjects with severe asthma, suggesting an alteration in the mast cell population distinctive for diseased airways, such as in severe asthma. The numbers and percentages of chymase-positive mast cells in severe asthma were greater than those found in control tissue, where the percentage of chymase-positive mast cells was in the range of 0–13%, consistent with previous studies (19, 20). In contrast, although the small control sample size limits comparison, no large differences were observed in the distribution of basic inflammatory cell types, including total mast cells, in the distal lung of subjects with severe asthma compared with control subjects. Additionally, in severe asthma, the correlation of chymase-positive mast cells with lung function was strongest in the small airway outer wall/alveolar attachments region. This region is likely to play a critical role in the balance of opposing airway wall and parenchymal tethering forces important to the maintenance of airway patency (21).

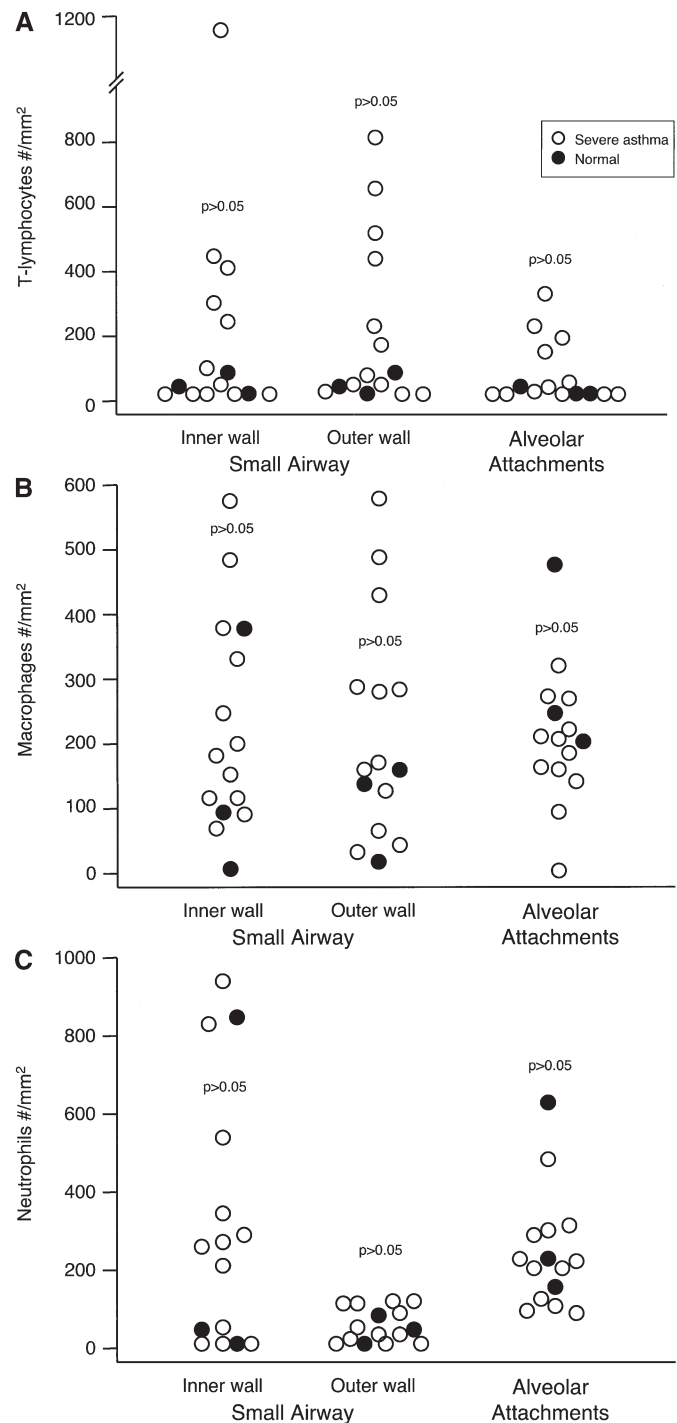


**Figure 6.** Distribution of lymphocytes (A), monocytes/macrophages (B), neutrophils (C), eosinophils (D), total mast cells (E), chymase-positive mast cells (F), and percentages of mast cells expressing chymase (G) in small airway regions and alveolar attachments of subjects with severe asthma (open circles) and normal subjects (filled circles).

Perhaps least expected, the relationship between chymase-positive mast cells and lung function was positive. The processes underlying the increase in chymase expression associated with better lung function in severe asthma are unknown and likely complex. It is conceivable that chymase-positive mast cells have a positive effect on airway remodeling through the ability of chymase to activate metalloproteinases, transforming growth

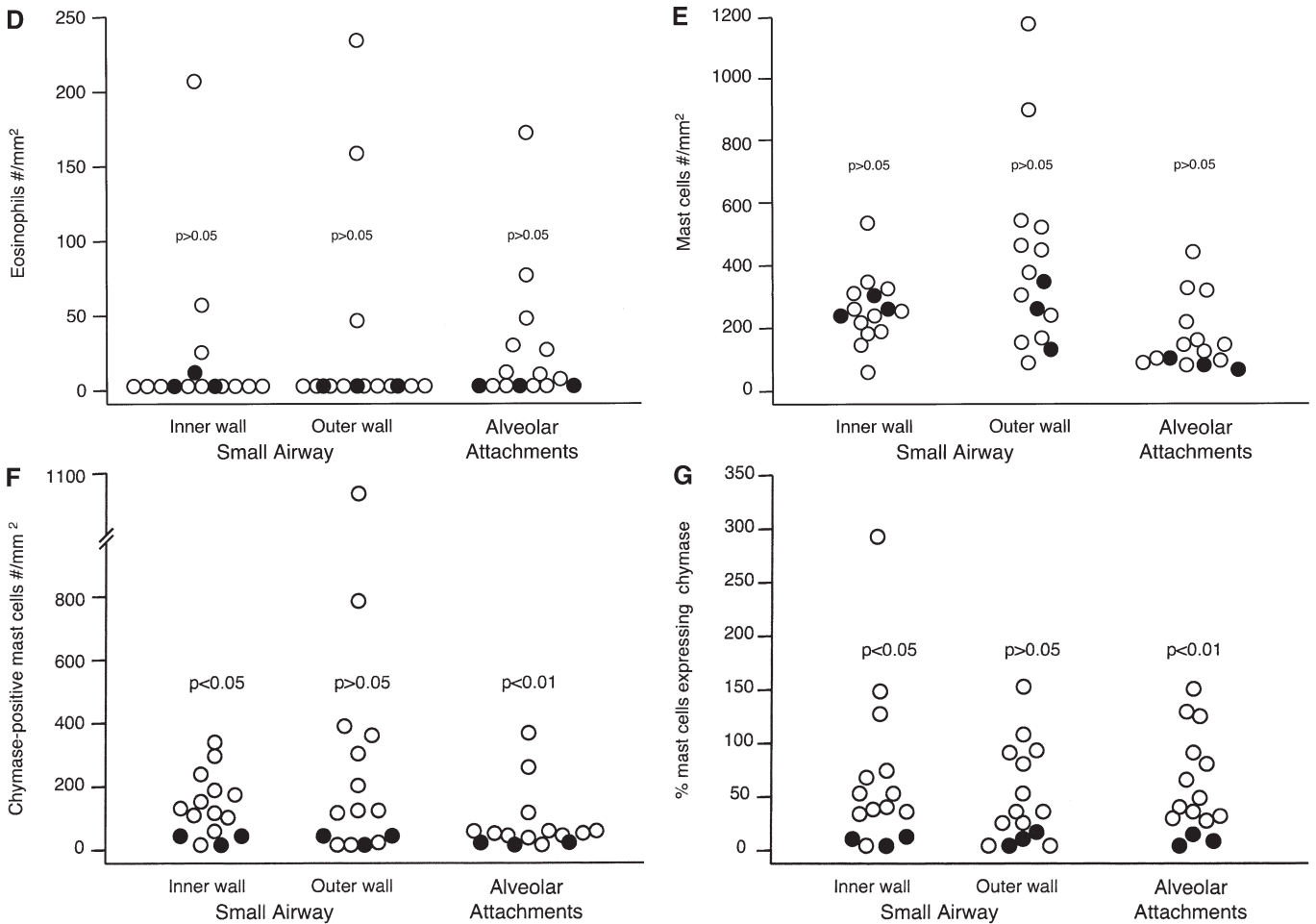


Figure 6. Continued.

factor- $\beta$ 1 and angiotensin II, and to inactivate thrombin and degrade matrix components such as fibronectin and vitronectin (22–24). Mast cells have also been demonstrated to induce fibroblast proliferation and collagen production *in vitro*, potentially contributing to greater deposition of extracellular matrix

in small airways, which may protect these small airways from collapse and improve lung function (25–28).

Finally, it is possible that the chymase-positive mast cell truly represents a different mast cell phenotype, the function of which goes beyond that of chymase alone. Little is known about what

TABLE 1A. CORRELATIONS BETWEEN INFLAMMATORY CELL COUNTS/PERCENTAGES AND LUNG FUNCTION MEASURES

| Region                           | Cell Type       | Lung Function         | Spearman Correlations (p value)<br>Between Lung Function and: |              |
|----------------------------------|-----------------|-----------------------|---------------------------------------------------------------|--------------|
|                                  |                 |                       | Cell #/mm <sup>2</sup>                                        | Cell %       |
| Large airway (n = 19)            | Total cells     | FEV <sub>1</sub>      | −0.48 (0.04)                                                  |              |
|                                  | Macrophages     | FEV <sub>1</sub>      |                                                               | 0.53 (0.02)  |
|                                  | Eosinophils     | FVC                   | −0.62 (0.006)                                                 | −0.53 (0.02) |
|                                  |                 | RV                    | 0.55 (0.02)                                                   | 0.59 (0.01)  |
|                                  |                 | FVC/SVC               | −0.58 (0.01)                                                  | −0.54 (0.02) |
| Small airway inner wall (n = 12) | Macrophages     | RV                    |                                                               | 0.77 (0.005) |
| Small airway outer wall (n = 12) | Eosinophils     | RV                    | −0.66 (0.03)                                                  | −0.66 (0.03) |
|                                  | MC <sub>T</sub> | FEV <sub>1</sub> /FVC | 0.71 (0.02)                                                   |              |
|                                  |                 | FEF <sub>25–75%</sub> |                                                               | −0.73 (0.02) |
| Alveolar attachments             | Macrophages     | FVC                   | −0.63 (0.04)                                                  |              |
|                                  | Eosinophils     | FEF <sub>25–75%</sub> | −0.67 (0.03)                                                  | −0.69 (0.03) |

Definition of abbreviations: FEF<sub>25–75%</sub> = forced expiratory flow at 25–75% of FVC; MC<sub>T</sub> = tryptase-positive mast cells; RV = residual volume; SVC = slow vital capacity.

Seventy-eight correlations were performed for each lung region. Only significant correlations (p < 0.05) are presented.

**TABLE 1B. CORRELATIONS BETWEEN CHYMASE-POSITIVE SUBSET OF MAST CELLS (MC<sub>TC</sub>) AND LUNG FUNCTION MEASURES**

| Region                           | Cell Type                           | Lung Function         | Spearman Correlations (p value)<br>Between Lung Function and: |                      |
|----------------------------------|-------------------------------------|-----------------------|---------------------------------------------------------------|----------------------|
|                                  |                                     |                       | Cell/mm <sup>2</sup>                                          | Cell %               |
| Small airway inner wall (n = 12) | MC <sub>TC</sub>                    | FEV <sub>1</sub> /FVC | 0.61 (0.046)                                                  |                      |
|                                  | MC <sub>TC</sub> /MC <sub>T</sub> % | FEF <sub>25-75%</sub> |                                                               | 0.75 (0.01)          |
| Small airway outer wall (n = 12) | MC <sub>TC</sub>                    | FVC                   | 0.61 (0.04)                                                   |                      |
|                                  | MC <sub>TC</sub>                    | FEV <sub>1</sub>      | <b>0.80 (0.003)</b>                                           |                      |
|                                  | MC <sub>TC</sub>                    | FEV <sub>1</sub> /FVC | <b>0.84 (0.001)</b>                                           |                      |
|                                  | MC <sub>TC</sub> /MC <sub>T</sub> % | FEV <sub>1</sub>      |                                                               | <b>0.88 (0.0003)</b> |
|                                  | MC <sub>TC</sub> /MC <sub>T</sub> % | FVC                   |                                                               | <b>0.65 (0.03)</b>   |
| Alveolar attachments (n = 12)    | MC <sub>TC</sub>                    | FEV <sub>1</sub>      | 0.67 (0.03)                                                   |                      |
|                                  | MC <sub>TC</sub>                    | FEV <sub>1</sub> /FVC | <b>0.82 (0.002)</b>                                           |                      |
|                                  | MC <sub>TC</sub> /MC <sub>T</sub> % | FEV <sub>1</sub>      |                                                               | <b>0.86 (0.0007)</b> |
|                                  | MC <sub>TC</sub> /MC <sub>T</sub> % | FVC                   |                                                               | <b>0.89 (0.0003)</b> |

Definition of abbreviations: FEF<sub>25-75%</sub> = forced expiratory flow at 25–75% of FVC; MC = tryptase-positive mast cells; MC<sub>TC</sub> = chymase-positive mast cells; RV = residual volume; SVC = slow vital capacity.

Seventy-eight correlations were performed for each lung region. Only significant correlations (p < 0.05) are presented. The correlations that remained significant after performing FDR procedure are printed in bold.

drives chymase expression *in vivo*, with no studies to suggest steroids enhance its expression. In fact, an *in vitro* study of mouse mast cells reported that glucocorticoids inhibited chymase expression, and an *in vivo* study of human proximal airway mast cells showed no change in (low) chymase expression after a 2-week steroid treatment period (29, 30). High concentrations of interleukin-4 and stem cell factor *in vitro*, as might be seen *in vivo* in severe asthma, can increase chymase production and could thereby alter the phenotype (31). Therefore, it is conceivable that the high chymase expression is due to an increase in the intracellular chymase/tryptase ratio from the value of 1:10 reported in normal lung mast cells (32). In subjects in whom chymase-positive mast cells could not be colocalized with tryptase-positive mast cells, those tryptase-negative cells may be similar to the previously described (and rare) chymase-positive/tryptase-negative mast cells. Such mast cells had no detectable tryptase by immunostaining, which is likely due to a low expression of tryptase rather than an absolute absence, because more sensitive electron microscopy studies of lung tissue mast cells report the presence of both enzymes in all mast cells (33–35).

In conclusion, total mast cells are increased in distal lung regions as compared with large airways. The increase in chymase-positive subset of these mast cells in severe asthma consistently correlated with better lung function, supporting a pivotal role for distal lung mast cells in modulating disease severity. These findings could hold considerable implications for both treatment approaches and long-term investigation of the disease. Clearly, further characterization of mast cells in peripheral airway tissue of subjects with asthma of various severities as well as *in vitro* studies of the function of the mast cell phenotypes are necessary to better understand their role in inflammation and the mechanisms involved in the protective effect observed in this study.

**Conflict of Interest Statement:** S.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; H.W.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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