

Chymase-positive mast cells play a role in the vascular component of airway remodeling in asthma

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Background: There is increasing evidence to support a role for total mast cells (MC_{TOT}) in the vascular component of airway remodeling in asthma. On the contrary, up to now, no study has addressed the role of chymase-positive mast cells (MC_{TC}) in microvasculature changes.

Objective: We sought to assess the role of MC_{TC} in the vascular component of airway remodeling in asthma.

Methods: We recruited 8 patients with mild-to-moderate asthma and 8 healthy volunteers as a control group. Fiberoptic bronchoscopy with endobronchial biopsy was successfully performed in all subjects. Immunostaining was performed for quantification of vessels, vascular endothelial growth factor (VEGF)-positive cells, MC_{TOT}, and MC_{TC}.

Results: Compared with those from healthy subjects, endobronchial biopsy specimens from asthmatic patients showed increased numbers of MC_{TOT} and MC_{TC} and VEGF⁺ cells ($P < .05$). In asthmatic patients the number of vessels and the vascular area was also greater than in healthy subjects ($P < .05$). Additionally, in asthmatic patients the number of MC_{TC} was significantly related to the vascular area ($r_s = 0.74$, $P < .01$) and to the number of VEGF⁺ cells ($r_s = 0.78$, $P < .01$). Moreover, a colocalization study revealed that MC_{TC} were a relevant cellular source of VEGF. Finally, a 6-week treatment with inhaled fluticasone propionate was able to reduce MC_{TC} numbers.

Conclusion: MC_{TC} can play a role in the vascular component of airway remodeling in asthma, possibly through induction of VEGF.

Clinical implications: Specific targeting of MC_{TC} might be a tool for treating vascular remodeling in asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Mast cells, bronchial asthma, vascular remodeling

Abbreviations used

MC_T: Tryptase-positive mast cells

MC_{TC}: Chymase-positive mast cells

MC_{TOT}: Total mast cells

VEGF: Vascular endothelial growth factor

The vascular component of airway remodeling is one of the aspects that significantly contributes to the alteration of the airway wall in asthma.^{1,2} The major structural and functional changes of the airway microcirculation include the proliferation of new vessels, increased blood flow and microvascular permeability, and edema formation in the airway wall.³

The cellular mechanisms responsible for these vascular changes are still unclear, although there is increasing evidence for mast cells as a source of proangiogenic factors. Mast cells can secrete several mediators involved in angiogenesis, such as the vascular endothelial growth factor (VEGF), histamine, the basic fibroblast growth factor, metalloproteinases, lipid-derived mediators, chemokines (IL-8 in particular), cytokines, and proteases.⁴

According to the protease content, human mast cells can be divided into those that express tryptase but not chymase (MC_T) and those that express tryptase and chymase (MC_{TC}).⁵ MC_T and MC_{TC} differ in tissue distribution and functional characteristics⁵ and, in asthmatic patients, also in the response to oral corticosteroids.⁶ Moreover, MC_{TC}, but not MC_T, express cathepsin G and carboxypeptidase A3 in their secretory granules, and CD88 on their surface, as the C5a receptor.⁷⁻⁹

Previous articles have supported the view that MC_T might be involved in the vascular changes of airway mucosa in asthmatic patients.¹⁰⁻¹² On the contrary, up to now, no study has been addressed to examine the role of MC_{TC} in the vascular component of airway remodeling in asthma.

There is evidence that human chymase can be involved in angiogenesis. Chymase converts angiotensin I to angiotensin II, which in turn is a potent angiogenic factor through VEGF induction.¹³ Chymase can also activate matrix metalloproteinase 9, which can lead to degradation

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of the extracellular matrix and basement membrane, migration of endothelial cells into the interstitial space, and the subsequent endothelial cell proliferation and differentiation into mature blood vessels.¹⁴

In this study we hypothesized that MC_{TC} would play a significant role in the vascular component of airway remodeling in asthma. To test this hypothesis, we evaluated the presence of MC_{TC} in the airways of patients with mild-to-moderate and clinically stable asthma against that seen in healthy control subjects and examined the relationship between MC_{TC} and the microcirculation changes in airway mucosa. Next, we also tested the effect of a short treatment course with inhaled fluticasone propionate on MC_{TC} counts.

METHODS

Subjects

Eight nonsmoking patients with mild-to-moderate asthma were recruited from the outpatient clinic of the Department of Clinical Sciences of Parma University. All had a well-documented history of asthma,¹⁵ and baseline FEV₁ had to be 70% or more of predicted value. All patients were free from asthma attacks in the previous 2 months and controlled their symptoms with inhaled salbutamol only. They did not receive corticosteroids for 6 months before the study and were free from respiratory infection in the 4 weeks preceding the study. The degree of asthma severity was assessed according to an asthma severity score based on symptoms, bronchodilator use, and daily peak expiratory flow variability measured during the 3 weeks before the methacholine challenge test day. Possible scores ranged from 0 to 12.¹⁶ Atopy was assessed by using skin prick tests with a battery of 10 common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Aspergillus fumigatus*, *Alternaria tenuis*, grass, *Parietaria* species, compositae, cupressaceae, cat dandruff, and dog dandruff). Eight healthy nonsmoking volunteers were enrolled as a control group.

The study was approved by the University of Parma ethical committee, and subjects provided written informed consent.

Spirometry and methacholine challenge

Baseline FEV₁ was measured in triplicate by using a flow-sensing spirometer connected to a computer for data analysis (Vmax 22; SensorMedics, Yorba Linda, Calif). Methacholine inhalation challenge was done according to the European Respiratory Society guidelines.¹⁷ Doubling increasing concentrations of methacholine from 0.03 to 32 mg/mL were delivered by using a dosimeter (output: 9 μ L per puff; Mefar, Brescia, Italy) and inhaled. Inhalations were interrupted when FEV₁ decreased by 20% from its postsaline value. The concentration provoking a 20% decrease in FEV₁ (PD₂₀ methacholine in μ mol) was determined by means of linear interpolation of the last 2 experimental points.

Fiberoptic bronchoscopy

Fiberoptic bronchoscopy was performed by a single bronchoscopist (R.D.), according to a previously described protocol.¹⁶ Premedication consisted of atropine (0.5 mg) and diazepam (10 mg), both administered by means of intramuscular injection. Each subject obtained local anesthesia by sucking a tetracaine tablet (20 mg) given 15 minutes before bronchoscopy. An additional aliquot of 2% lignocaine was aerosolized into the upper airways and applied topically to the pyriform sinuses and vocal cords to prevent coughing and as a local anesthetic. Nebulized salbutamol (1.25 mg) and ipratropium bromide (0.25 mg) were inhaled 5 minutes before bronchoscopy

(Model 1T10; Olympus, Tokyo, Japan). Three to 5 mucosal biopsy specimens were taken at the subcarinae in the middle and lower lobes of the right lung. Administration of nebulized salbutamol and ipratropium bromide was repeated after bronchoscopy, when necessary. Patients were closely monitored after bronchoscopy in the outpatient clinic and were discharged when the effects of sedation disappeared and lung function returned to the baseline values.

All the asthmatic patients underwent a second fiberoptic bronchoscopy with bronchial biopsy after a 6-week course of medium-dose inhaled fluticasone propionate.

Biopsy processing

Mucosal biopsy specimens were immediately transferred into ice-cold acetone containing the protease inhibitor iodoacetamide (20 mmol/L) and phenylmethylsulfonyl fluoride (2 mmol/L) for fixation; they were then stored at -20°C for 24 hours and processed into the water-soluble resin glycolmethacrylate (Polysciences, Northampton, United Kingdom) for embedding.¹⁸

Sections of 2 μ m were cut and incubated with primary antibodies for 16 to 20 hours overnight at room temperature. We used mAbs against type IV collagen to outline the endothelial basement membrane (1:400; Novocastra Labs, Newcastle, United Kingdom); trypsinase (1:200; Dako, Glostrup, Denmark) and chymase (1:200; Novocastra Labs) to identify total mast cells (MC_{TOT}) and MC_{TC}, respectively; and VEGF (1:50; NeoMarkers, Fremont, Calif) to recognize the 121, 165, and 189 isoforms of VEGF. After washing in Tris-buffered saline, the sections were incubated for 2 hours with biotin-conjugated secondary antibody. We used a streptavidin biotin-peroxidase complex detection system with the second-stage biotinylated Ig F(ab')₂ fragments raised against the appropriate animal. In particular, all the mAbs were labeled with biotinylated anti-mouse IgG (Vector Labs, Burlingame, Calif). Light microscopy was performed with a Leica microscope (Leica DMLB, Werzlar, Germany) at $\times 1000$ magnification.

As previously described,¹¹ the number of vessels, MC_{TOT}, MC_{TC}, and VEGF⁺ cells were counted in all nonoverlapping high-power fields in the lamina propria. The latter is defined as a zone 100 μ m below the epithelial basement membrane and expressed as the number of vessels and cells per square millimeter of lamina propria. In each section all available nonoverlapping high-power fields with intact tissue covered by intact basement membrane were examined. Vascular area was expressed as a percentage of the area of the assessed lamina propria. The mean size of vessels was estimated by dividing the total vascular area by the total number of vessels. Final values represent the mean of at least 2 sections from 2 different biopsy specimens in each subject. A single observer who had no knowledge of patient characteristics performed 3 replicate measurements of morphometric parameters by using an image analysis system (Image-Pro Plus; MediaCybematics, Inc, Silver Spring, Md). An average of 8 high-power fields were examined for each subject included in the study. The mean coefficient of variation for repeated measurements, obtained by reassessing the same biopsy sections with an interval of at least 1 week, was 10% for number of vessels and vascular area, 9% for VEGF⁺ cells, 8% for MC_{TOT}, and 9% for MC_{TC}.

Statistical analysis

Values are presented as means $\pm$ SDs, unless otherwise specified. PD₂₀ methacholine values were log-transformed before analysis. The unpaired *t* test, the Mann-Whitney rank sum test, and the χ^2 test were used for comparison between patients with asthma and control subjects, when appropriate. Changes in functional and morphologic data before and after treatment were calculated by using the Wilcoxon matched pairs test. Relationships were estimated by using the Spearman rank correlation coefficient (*r_s*). A *P* value of .05 or less was taken as significant.

TABLE 1. Characteristics of asthmatic and healthy control subjects

	Asthmatic subjects (n = 8)	Control subjects (n = 8)
Age (y)	26 ± 8	25 ± 3
Sex (F/M)	2/6	4/4
Atopy (yes/no)	8/0	0/8
Duration of disease (y)	10 ± 5	—
FEV ₁ (% predicted)	110 ± 20	108 ± 8
PD ₂₀ methacholine (μmol)	1.7 ± 1.9	>40
Asthma severity score (0-12)	6 (5-10)	—

RESULTS

Characteristics of asthmatic patients and healthy control subjects are reported in Table 1. According to the asthma severity score, patients with asthma had persistent mild-to-moderate asthma (score range, 5-10; median value, 5). All patients were atopic.

Fiberoptic bronchoscopy with endobronchial biopsy was performed successfully and was well tolerated in all subjects. Patients with asthma significantly differed from healthy volunteers in MC_{TOT} (70 ± 14 vs 35 ± 9 cells/mm², $P < .05$), MC_{TC} (18 ± 12 vs 4.5 ± 1.2 cells/mm², $P < .05$), VEGF⁺ cells (103 ± 48 vs 35 ± 21 cells/mm², $P < .05$), number of vessels (211 ± 43 vs 153 ± 23 vessels/mm², $P < .01$), and vascular area ($4.8\% \pm 1.5\%$ vs $3.0\% \pm 0.7\%$, $P < .05$). There was no significant difference in mean vessel size in patients with asthma compared with control subjects (242 ± 48 vs 203 ± 31 μm²). In asthmatic patients the ratio of the MC_{TC} count to the MC_{TOT} count was 0.25 ± 0.14 , whereas in healthy volunteers the ratio was 0.13 ± 0.04 .

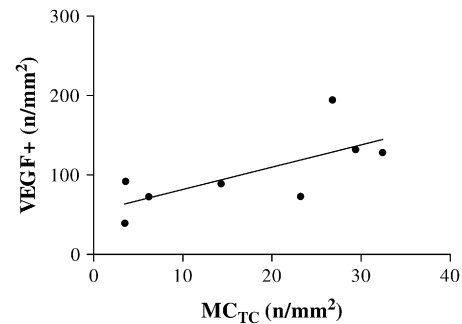
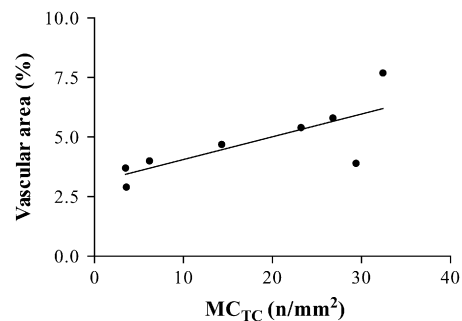
In patients with asthma, the MC_{TC} number significantly correlated with the number of VEGF⁺ cells ($r_s = 0.78$, $P < .01$, Fig 1) and the vascular area ($r_s = 0.74$, $P < .01$, Fig 2) and tended to be related to the vessel number ($P = .08$). Moreover, a significant positive correlation was observed between the MC_{TC}/MC_{TOT} ratio and the PD₂₀ methacholine values ($r_s = 0.76$, $P < .05$).

Examination of serial biopsy sections was performed, and comparison of adjacent sections showed that in the lamina propria a median of 28% (range, 19.8% to 42.6%) of MC_{TC} colocalized with VEGF⁺ cells (Fig 3).

After the 6-week course of inhaled fluticasone propionate with a daily average dose of 600 ± 370 μg, the numbers of MC_{TOT} (35 ± 15 vs 70 ± 14 cells/mm², $P < .05$), MC_{TC} (11 ± 7 vs 18 ± 12 cells/mm², $P < .05$), and VEGF⁺ cells (72 ± 30 vs 103 ± 48 cells/mm², $P < .05$) were significantly reduced compared with baseline values. By contrast, the number of vessels and the vascular area were not affected by steroid treatment.

DISCUSSION

In this study we examined the relationship between MC_{TC} numbers in the large airways of patients with mild-

**FIG 1.** Correlation between the number of MC_{TC} and the number of cells expressing VEGF in patients with asthma ($r_s = 0.78$, $P < .01$).**FIG 2.** Correlation between the number of MC_{TC} and the vascular area in patients with asthma ($r_s = 0.74$, $P < .01$).

to-moderate asthma and changes to the airway vascular bed. We found that MC_{TC} numbers were increased in the airways of asthmatic patients compared with those of control subjects and were significantly associated with both the area occupied by vessels and VEGF expression. Finally, we observed that a short treatment with a medium dose of inhaled fluticasone propionate was able to reduce MC_{TC} numbers in the airways of asthmatic patients.

Although it is well known that MC_T might have a role in the vascular remodeling of asthmatic airways,^{3,10} this is the first evidence of a relationship between MC_{TC} and airway microvasculature in asthma. Although the relation does not infer causality, this finding could be relevant to the airway angiogenesis. In disease conditions other than asthma, previous studies have showed that MC_{TC} can play an angiogenic role.^{19,20} Indeed, high numbers of MC_{TC} in close contact with endothelial cells are present in atopic dermatitis skin lesions and are associated with upregulation of proangiogenic factors, such as VEGF, TNF-α, and metalloproteinase 2.²¹ In the field of respiratory medicine, a recent contribution²⁰ has supported the importance of MC_{TC} in the angiogenesis of lung cancer. In that study the authors found that the number of MC_{TC} was significantly increased in tissue from non-small cell lung cancer compared with normal lung parenchyma. Moreover, they demonstrated a significant correlation between MC_{TC} counts and microvessels. Altogether, these results confirm the view that angiogenesis is a multistep process regulated by a large number of mediators, some

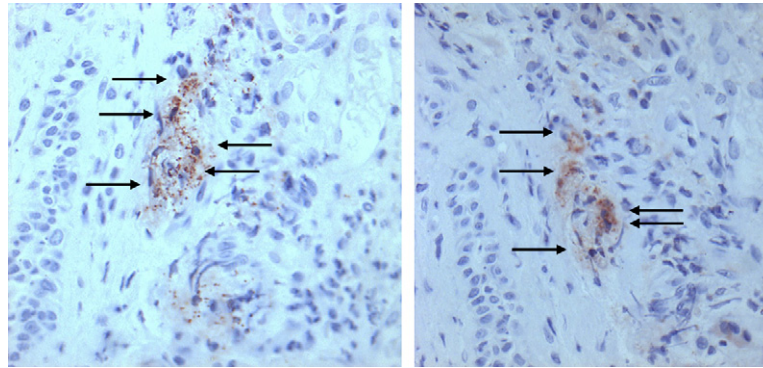


FIG 3. Microphotograph showing sequential 2- μ m sections of bronchial mucosa from an asthmatic patient stained with antibodies to MC_{TC} (left) and VEGF (right). Arrows show cells stained with both antibodies, indicating colocalization.

having an angiogenic activity and some being able to induce the degradation of the extracellular matrix and basement membrane, which is considered a prerequisite for the development of new vessels.

Our results are in line with the observations of Balzar et al,²² who examined the distribution of different inflammatory cells in the airways of patients with severe asthma, analyzing both the large and the distal airways. The authors could detect MC_T and MC_{TC} in all the districts, particularly in small airway regions. However, the small size of the control group in that study did not allow the authors to draw definite conclusions on the increase of MC_{TC} numbers in patients with asthma. In our study we extend those results by showing an increased number of MC_{TC} in the large airways of asthmatic subjects compared with numbers in a proper control group of healthy volunteers.

The magnitude of chymase expression and the MC_{TC}/MC_{TOT} ratio we observed were similar to those reported by previous studies in both asthmatic patients and healthy control subjects²²⁻²⁵ that analyzed the MC_{TC}/MC_T ratio. This is not surprising because MC_T were found to be the mast cell–predominant subset both in bronchial mucosa and alveoli.²²⁻²⁵ It is noteworthy that in our study a substantial subset of mast cells (about 25%) expressed chymase, thus identifying a specific mast cell phenotype with an important effect on vascular remodeling. The contribution of chymase in mast cell–mediated angiogenesis was investigated by Muramatsu et al²⁶ in a hamster system, which expresses an abundant variety of chymases. The authors reported that local injection of a potent mast cell activator, the compound 48/80, induced angiogenesis in a time- and dose-dependent manner, and chymase, but not tryptase, inhibitors prevented the angiogenic response. It is of note that the authors did not show whether the compound 48/80 effect was mast cell dependent.²⁶ In another study the same authors suggested a possible involvement of a chymase–angiotensin–dependent pathway in angiogenesis, possibly through the release of growth factors, such as basic fibroblast growth factor and VEGF.⁴ Indeed, our results support this hypothesis because although we cannot exclude other sources of VEGF, our colocalization analysis demonstrated that MC_{TC} are a

relevant source of VEGF in asthmatic airways and that the number of MC_{TC} is significantly related to the area occupied by vessels.

In our study we also observed that the MC_{TC}/MC_{TOT} ratio was directly related to bronchial responsiveness to methacholine, in agreement with Balzar et al,²² who reported a positive relationship between lung function and the MC_{TC}/MC_T ratio in distal regions of the lung. Although the relationship between mast cell tryptase and bronchial hyperresponsiveness has been previously examined, the effect of chymase has been less extensively studied.²⁷⁻²⁹ It is known that reactivity to histamine in both proximal and distal bronchi is significantly increased by a previous incubation with tryptase, an effect that is completely abolished in the presence of protease inhibitors.²⁹ Unexpectedly, our own study and the study by Balzar et al²² seem to indicate that chymase might have a positive effect on lung function, and it has been therefore proposed that MC_{TC} might have a protective role. On the other hand, it is also possible that the low numbers of MC_{TC} in the submucosa of asthmatic patients with the highest degree of hyperreactivity might reflect a preferential recruitment of these cells to the airway smooth muscle bundles, where they can exert contractile functions.³⁰ It is of note that in our study the absolute number of MC_{TC} was correlated to increased vascularity, but not to hyperreactivity, which was related to the MC_{TC}/MC_{TOT} ratio, and we cannot therefore determine the specific role of MC_{TC} or MC_{TOT} in this effect. Given the complexity of the relationship between chymase expression and bronchial hyperresponsiveness, a specific evaluation of the underlying mechanisms deserves further investigation.

Up to now, there are few studies that have evaluated the therapeutic effect of steroids on MC_{TC} numbers *in vivo*. In our study we provided the first evidence of an MC_{TC} reduction by corticosteroid treatment. Our results differ from those of previous reports.^{6,31} Bentley et al,⁶ evaluating the effects of prednisolone treatment on inflammatory cells infiltrating the bronchial mucosa of 9 patients with moderate-to-severe asthma, could demonstrate a reduction in MC_T cells but not MC_{TC} cells. Similarly, Juliusson et al³¹ examined, in the nasal mucosa of 6

patients with birch pollen allergy, the topical effects of corticosteroid treatment on mast cell number and found that MC_T, but not MC_{TC}, numbers were decreased. In both studies the authors explained the lack of effect of steroids on MC_{TC} numbers because of the scarce numbers of MC_{TC} detected at baseline conditions. In our study steroid treatment was able to significantly reduce the number of MC_{TC} and VEGF⁺ cells but not the number of vessels and the vascular area. These results are probably due to the use of a medium dose instead of a high dose of inhaled steroids.^{11,32}

We should acknowledge that unlike previous reports,^{22,23} in our study we did not perform double immunostaining for mast cell detection. As previously reported by Irani et al,²³ the deposition of the brown peroxidase-dependent reaction product in MC_{TC} cells stained with mAb B7 blocks the subsequent staining of tryptase present in these cells by the alkaline phosphatase-conjugated anti-tryptase mAb G3. As a result, only chymase-negative MC_T cells bind G3 and are stained blue with the alkaline phosphatase substrate, whereas MC_{TC} cells remain brown, allowing a clear distinction between the 2 types of mast cells.^{23,33} However, Schwartz,³⁴ who recently focused on the tissue detection of mast cells, considered the single immunostaining as a suitable technique to detect MC_{TOT} by using antitryptase immunocytochemistry and MC_{TC} by using antichymase immunocytochemistry. We also should acknowledge that phenylmethylsulfonyl fluoride could inhibit chymase and tryptase proteases because they are serine proteases. We used glycolmethacrylate for embedding biopsy samples, and in this case the cold acetone fixation seems to be a good compromise.¹⁸ Furthermore, we found that the percentage of MC_{TC} cells was not dissimilar to that reported by studies that used Carnoy's fluid.²²⁻²⁵

In summary, we found that in asthmatic patients increased chymase-expressing mast cells might be associated with an increased vascularity in airway mucosa. Our results further support the view that in bronchial asthma mast cells play a key role in the vascular component of airway remodeling.

REFERENCES

- Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;161:1720-45.
- McDonald DM. Angiogenesis and remodeling of airway vasculature in chronic inflammation. *Am J Respir Crit Care Med* 2001;164(suppl):S39-45.
- Wilson J. The bronchial microcirculation in asthma. *Clin Exp Allergy* 2000;30:51-3.
- Norby K. Mast cell and angiogenesis. *APMIS* 2002;110:355-71.
- Metcalfe DD, Baram D, Mekori Y. Mast cells. *Physiol Rev* 1997;77:1033-79.
- Bentley AM, Hamid Q, Robinson DS, Schotman E, Meng Q, Assoufi B, et al. Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. *Am J Respir Crit Care Med* 1996;153:551-6.
- Schechter NM, Irani AA, Sprows JL, Abernethy J, Wintroub B, Schwartz LB. Identification of a cathepsin G-like proteinase in the MCTC type of human mast cell. *J Immunol* 1990;145:2652-61.
- Irani AA, Goldstein SM, Wintroub BU, Bradford T, Schwartz LB. Human mast cell carboxypeptidase—selective localization to MC_{TC} cells. *J Immunol* 1991;147:247-53.
- Oskeritzian CA, Zhao W, Min HK, Xia HZ, Pozez A, Kiev J, et al. Surface CD88 functionally distinguishes the MC_{TC} from the MC_T type of human lung mast cell. *J Allergy Clin Immunol* 2005;115:1162-8.
- Wilson JW, Hii S. The importance of the airway microvasculature in asthma. *Curr Opin Allergy Clin Immunol* 2006;6:51-5.
- Chetta A, Zanini A, Foresi A, D'Ippolito R, Tipa A, Castagnaro A, et al. Vascular endothelial growth factor upregulation and bronchial wall remodelling in asthma. *Clin Exp Allergy* 2005;35:1437-42.
- Blair JR, Meng H, Marchese MJ, Ren S, Schwartz LB, Tonnesen MG, et al. Human mast cells stimulate vascular tube formation—tryptase is a novel, potent angiogenic factor. *J Clin Invest* 1997;99:2691-700.
- Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol Rev* 2000;52:11-34.
- Kanbe N, Tanaka A, Kanbe M, Itakura A, Kurosawa M, Matsuda H. Human mast cells produce matrix metalloproteinase 9. *Eur J Immunol* 1999;29:2645-9.
- American Thoracic Society. Standards for diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
- Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. *Chest* 1997;111:852-7.
- Sterk PJ, Fabbri LM, PhH Quarnier, Cockcroft DW, O'Byrne PM, Anderson SD, et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. *Eur Respir J* 1993;6(suppl 16):53-8.
- Britten KM, Howarth PH, Roche WR. Immunohistochemistry of resin sections, a comparison of resin embedding techniques for small mucosal biopsies. *Biotech Histochem* 1993;68:271-80.
- Nagata M, Shijubo N, Walls AF, Ichimiya S, Abe S, Sato N. Chymase-positive mast cells in small sized adenocarcinoma of the lung. *Virchows Arch* 2003;443:565-73.
- Ibaraki T, Muramatsu M, Takai S, Jin D, Maruyama H, Orino T, et al. The relationship of tryptase- and chymase-positive mast cells to angiogenesis in stage I non-small cell lung cancer. *Eur J Cardiothorac Sur* 2005;28:617-21.
- Groneberg DA, Bester C, Grützkau A, Serowka F, Fischer A, Henz BM, et al. Mast cells and vasculature in atopic dermatitis—potential stimulus of neoangiogenesis. *Allergy* 2005;60:90-7.
- Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am J Respir Crit Care Med* 2005;171:431-9.
- Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci U S A* 1986;83:4464-8.
- Matin R, Tam EK, Nadel J, Caughey GH. Distribution of chymase-containing mast cells in human bronchi. *J Histochem Cytochem* 1992;40:781-6.
- Hamada H, Terai M, Kimura H, Hirano K, Oana S, Niimi H. Increased expression of mast cell chymase in the lungs of patients with congenital heart disease associated with early pulmonary vascular disease. *Am J Respir Crit Care Med* 1999;160:1303-8.
- Muramatsu M, Katada J, Hattori M, Hayashi I, Majima M. Chymase mediates mast cell-induced angiogenesis in hamster sponge granulomas. *Eur J Pharmacol* 2000;402:181-91.
- Foresi A, Leone C, Pelucchi A, Mastropasqua B, Chetta A, D'Ippolito R, et al. Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997;100:58-64.
- Johnson PR, Ammit AJ, Carlin SM, Armour CL, Caughey GH, Black JL. Mast cell tryptase potentiates histamine-induced contraction in human sensitized bronchus. *Eur Respir J* 1997;10:38-43.
- Berger P, Compton SJ, Molimard M, Walls AF, N'Guyen C, Marthan R, et al. Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. *Clin Exp Allergy* 1999;29:814-22.
- Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002;346:1699-705.

31. Juliusson S, Aldenberg F, Enerbäck L. Proteinase content of mast cells of nasal mucosa: effects of natural allergen exposure and of local corticosteroid treatment. *Allergy* 1995;50:15-22.
32. Chetta A, Zanini A, Foresi A, Del Donno M, Castagnaro A, D'Ippolito R, et al. Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. *Am J Respir Crit Care Med* 2003;167:751-7.
33. Irani AA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB. Detection of MCt and MCTC of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. *J Histochem Cytochem* 1989;37:1509-15.
34. Schwartz LB. Analysis of MC(T) and MC(TC) mast cells in tissue. *Methods Mol Biol* 2006;315:53-62.