



Differential accumulation of pulmonary and cardiac mast cell-subsets and eosinophils between fatal anaphylaxis and asthma death A postmortem comparative study

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Abstract

The distribution profile of infiltrated mast cell-subpopulations and eosinophils in the lung and heart sections of the patients who died of severe allergic hyperresponsiveness, was investigated. Four study groups were designed comprising 9 cases who died in systemic anaphylaxis (Group I), 10 asthmatic individuals whose death were assigned to acute and severe bronchial asthma (Group II), 10 asthmatic cases who died from non-immunological diseases (Group III). Twenty consecutive autopsies of non-allergic subjects who died of unnatural causes (Group IV) served as control group in this study. Utilizing antibodies against human tryptase and chymase and a double immunohistochemical staining method, we distinguished successfully all three subsets of mast cells (MC), MC-TC (containing both tryptase and chymase), MC-T (containing only tryptase) and MC-C (containing only chymase) types, subdivided on the basis of the protease compositions of their secretory granules. In order to immunostaining eosinophils, we used antibody to major basic protein as a marker. We also measured postmortem blood tryptase, specific and total serum IgE. The intriguing finding of this study was the marked differences of cellular composition in the lung between fatal anaphylaxis and asthma death. Significant augmentation of MCs infiltrated in lung and heart sections of anaphylaxis patients and drastic infiltration of bronchial eosinophils in asthmatic death and consequent release of their related inflammatory mediators might explain the differential expression of the associated symptoms in these two groups. The anaphylactic deaths did show neither emphysema nor significant mucous bronchial secretions whereas all asthmatic deaths did. The degree of pulmonary congestion and edema was also more severe in anaphylaxis. This corresponded with the histological findings and the location and number of mast cell-subsets and eosinophils in the different compartments of the lungs. We have demonstrated that the third type of mast cell MC-C is only found in the lungs in anaphylactic deaths. The practical consequence of our study will be that it is now possible to confirm a suspicion of anaphylaxis death not only by measurements of serum mast cell tryptase, but also by immunohistochemical methods.

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1. Introduction

Allergic responses generally are classified as immediate, late phase or chronic. When an allergen is encountered systemically, anaphylaxis may occur. Anaphylaxis is a syndrome with varied mechanisms, clinical presentations and severity and is an acute life-threatening reaction, which results from a sudden systemic release of mast cell and basophil mediators.

Anaphylaxis often produces symptoms within 5–30 min after exposure and usually is mediated by an immunologic mechanism through IgE-dependent events, but not always so [1]. The more rapid the onset of the signs and symptoms of anaphylaxis after exposure to an offending stimulus, the more likely the reaction will be severe and life-threatening [2]. Urticaria and angioedema are the most common manifestations of anaphylaxis and occur as the initial signs and symptoms [3]. The most common identifiable causes of anaphylaxis are foods, medications, insect stings and drugs.

Classically, asthma was divided into two major categories: extrinsic (allergy, atopic) asthma, referring to those cases where bronchoconstriction was believed to be induced by inhaled

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allergen and intrinsic (idiopathic, non-atopic) asthma, which was considered unrelated to immune mechanisms [4]. Allergic asthma is a chronic disease of the respiratory tract, it is characterized by relapsing-remitting episodes of reversible airway obstruction. Eosinophilia, lung inflammation, elevated serum IgE, mucus hypersecretion and hyperreactive airways are the vital signs of allergic asthma. A severe asthma attack may come with a sudden or slow onset. Clinically, sudden-onset asthma attacks are defined by a sudden development of airway obstruction. Slow-onset asthma attacks are associated with a progressive but slow clinical and functional deterioration [4]. Severe asthma can have fatal consequences. Because mast cell stabilizing drugs have little value in treating chronic asthma, it has been assumed that mast cells do not contribute to later events in the pathophysiology of this disease.

Activation of mast cells is triggered by either the interaction of allergen with membrane bound IgE so called IgE-mediated reaction (anaphylactic reaction) or through direct binding of agent to the cell surface receptor initiating a cascade of non-IgE mediated activity (anaphylactoid reaction) [5]. These interactions lead to the release of a variety of chemical mediators that are an integral part of the allergic responses. The mast cell is able to release potent inflammatory mediators, which potentially damage and activate the bronchial epithelium, connective tissues and contribute to airway wall remodelling [3]. Histamine, tryptase and chymase are important markers of mast cell activations. Histamine is unsuitable for the postmortem diagnosis of anaphylaxis because of its very short half-life in the circulation whereas, tryptase [6] and chymase [7] are stable postmortem. After their maturation in peripheral tissue, MCs develop into three subsets of cell: MC-T, mast cells predominantly express the protease tryptase. This subset generally is localized to mucosal surface, often in close proximity to T cells, especially T-helper 2 type [8]. MC-TC, mast cells express tryptase, chymase, carboxypeptidase and cathepsin G. It tends to predominate in connective tissue [9]. A third type of these cells containing chymase and exclude tryptase (MC-C) has been observed, but the function and tissue localization of this subset is mainly unknown [10]. Activated eosinophils and eosinophil granule proteins such as major basic protein (MBP) and eosinophil cationic protein (ECP), are involved in the pathology of allergy and asthma [11]. The cationic nature of these molecules would indirectly affect microvascular permeability *in situ* by stimulating perivascular mast cells to release vasoactive and proinflammatory mediators [12]. Eosinophils might play either a proinflammatory role, e.g. release of cytotoxic granule-associated proteins or an anti-inflammatory role, e.g. metabolism of vasoactive mediators [13].

Despite recent advances in the understanding of the pathogenesis and pathophysiology of anaphylaxis and severe asthma, how the hyperresponsive state is acquired is not clearly understood yet, but it is likely to result from several cellular and molecular effectors mechanisms. Therefore, the purpose of this project was to compare the distribution of mast cell subpopulations and eosinophils immunohistochemically in the lung and heart of individuals who died in anaphylaxis

(Group I), in asthmatic individuals whose deaths were either assigned to acute and severe bronchial asthma (Group II) or to non-immunological causes (Group III) and compare those with a control group (Group IV) of non-allergic individuals who died of unnatural causes. Moreover, we wanted to find out if the number of mast cells and eosinophils correlated with the concentration of mast cell tryptase in serum.

2. Materials and methods

2.1. Subjects

The study subjects in Group I included nine autopsy cases of certain deaths where the autopsies were made during 1991–2004 by medical examiners at our Forensic Medicine Department and at the Department of Forensic Medicine in Stockholm, Sweden. The criteria for Group I cases were as follows: elevated level of femoral β -tryptase with the cut-off value set of 10 $\mu\text{g/l}$ [14] and $\alpha + \beta$ -tryptase 45 $\mu\text{g/l}$ [15], elevated total serum (s)-IgE or specific s-IgE and/or a temporal relationship between death and a preceding challenging incident, e.g. the injection or ingestion of a therapeutic agent, ingestion of certain foods or bee sting. None of the patients had a history of mastocytosis and showed no dermatological efflorescences suggesting urticaria pigmentosa.

Case 1: Female 21 years with diagnosed animal allergy and asthma was found unconscious in her apartment. The autopsy showed no specific findings apart from low degree of mucous secretion in the bronchi and histologically infiltrates of neutrophils and eosinophils. The respiratory changes were not as severe as to ascribe the death to acute asthma. Mast cell tryptase was not possible to analyze at that time (1991).

Case 2: Male 44 years with respiratory infection was prescribed penicillin-V. About 20 min after ingestion of the first dose, he fell ill and was found dead in a toilet facility. At autopsy, non-specific pulmonary stasis and edema was the only finding. S-tryptase and s-IgE for different penicillins were analyzed.

Case 3: Male 45 years was found dead in his car that was parked at the side of a country road. He had no history of asthma or allergy. At autopsy no signs of asthma or other diseases were found. Analyses of mast cell tryptase, total serum IgE, s-IgE against bee and wasp venom as well as food-mix (a panel of food-allergens) and Phadiatop (a panel of air-borne allergens) were performed.

Case 4: Male 47 years found dead in the bathroom at midnight after going to bed at 22.00. He had known allergy against mites and asthmatic bronchitis. He had previously had several attacks of shortness of breath, nasal obstruction, tickling sensations in hands and feet, tachycardia and a feeling of total exhaustion afterwards. At autopsy was found pulmonary congestion but no signs of asthmatic bronchitis and slight cardiac hypertrophy without significant coronary artery disease. Analyses of mast cell tryptase, total s-IgE and s-IgE for different mites were done. Also mite-allergens were sought for by vacuum cleaning of his mattress.

Case 5: Male 55 years was found dead on the lawn at his house. He had not complained of any previous symptoms. He had no history of asthma or allergy. At autopsy a severe congestion of the lungs was found and moderate bronchial, mucous secretion. Histologically a moderate bronchitis with infiltrated eosinophils was seen. Analyses of tryptase and s-IgE food-mix and Phadiatop were performed.

Case 6: Male 60 years with a history of alcohol abuse trying to get rid of a wasps nest was stung several times and died after 20 min. Had laryngeal edema and pulmonary congestion but no signs of asthma. S-tryptase and s-IgE for wasp and bee venom was done.

Case 7: Male 17 years with no history of allergy or asthma had a meal at a kebab stand and collapsed in the street a short time afterwards. At autopsy no specific organ changes were seen and no signs of bronchial secretion. The stomach content comprised kebab meat, paprika and other non-identifiable vegetables. Analyses of s-tryptase and s-IgE for different food allergens were performed.

Case 8: Male 23 years old was given penicillin-V for a skin infection. Autopsy findings and microscopy revealed acute bronchitis and incipient bronchopneumonia without signs of asthma, pulmonary congestion and slight cerebral edema. S-tryptase and total s-IgE were analyzed.

Case 9: Male 55 years with symptoms of kidney stone was injected with radio-opaque dye in order to perform X-ray investigation. Within minutes he collapsed and resuscitation followed for about an hour without success. The autopsy findings were a concretion in the right ureter with pyelitis, brain edema, pulmonary congestion and edema. There were no microscopical signs of asthma but a few microthrombi in the capillaries. S-tryptase and total s-IgE was analyzed.

The study subjects in Group II include 10 deaths assigned to acute bronchial asthma and in Group III consist of 10 cases with a history of asthmatic bronchitis that died from other non-immunological causes such as myocardial infarction and alcohol intoxication. The control cases (Group IV) were 20 consecutive cases collected during the same period, 55 years of age or below and all died from known causes, i.e. suicidal hanging, drowning, brain damage or traffic accident and all with no history of allergy. We took the collected medical and other data from police and hospital records into consideration, with special reference to possible heredity for allergy and exposure to allergens. The mean time between death and autopsy was less than 5 days. The majority of the bodies had been stored at 4 °C within 24 h after death.

2.2. Determination of tryptase, specific- and total-IgE

Femoral blood was sampled in test tubes without additives. They were centrifuged and the serum was frozen at –20 °C until analysis. β -tryptase was determined with a commercial kit (Pharmacia Tryptase RIAC, Pharmacia Diagnostic, Uppsala, Sweden) a radioimmunoassay with a lower detection limit of 2 μ g/l. α - and β -Tryptase were measured with Tryptase FEIA (Pharmacia Diagnostics, Uppsala, Sweden). Total serum-IgE was measured with Pharmacia CAP-system IgE-FEIA and allergen specific IgE with Pharmacia CAP system specific IgE-FEIA (Pharmacia Diagnostics, Uppsala, Sweden). The analyses were done at the Laboratory of Clinical Immunology and Transfusion Medicine, Karolinska sjukhuset, Stockholm, Sweden. The test for mast cell tryptase available in the Sweden before 1998 detected β -tryptase and since then the most widely used test does not distinguish between α -tryptase and β -tryptase.

2.3. Sequential immunohistochemical labelling

Sequential double-labelling with anti-tryptase monoclonal antibody (Mab) and anti-chymase Mab, modified from a method of Irani et al. [16], was used to display differentially stained MC-TC, MC-T and MC-C in the same tissue sections. Sections labelled with the antibodies were successively stained with the appropriate chromogenes to distinguish tryptase and/or chymase containing cell by developing different colour. Exclusion of streptavidine and substitution of this step with a secondary antibody that directly conjugated to poly-peroxidase molecule improved the staining. Using this technique we could differentiate between MC-TC cells containing both tryptase and chymase in secretory granules, MC-T and MC-C cells lacking chymase and tryptase, respectively.

For each case, four sequential sections from the left and right ventricular wall and septum, along with four lung specimens from four of the five lobes of both lungs, were fixed in 4% phosphate-buffered formalin, embedded in paraffin and cut into sections of 4 μ m. All sections were deparaffined and rehydrated with graded alcohols and finally in distilled water. All slides were incubated with 3% H₂O₂ in Phosphate-buffer saline (PBS) for 10 min at room temperature (RT) and were investigated with a retrieval technique: pretreated 20 min with trypsin digestion (0.3%, PH 7.8, RT). After that, 20 min treatment with blocking solution to block unspecific bindings. Monoclonal antibodies were applied to the sections at a dilution of 1:100 for anti-tryptase and chymase (NOVO Castra Laboratories LTD., UK) and 1:40 for MBP as an eosinophil marker (Nordic Biosite AB, Täby, Sweden) and all incubated at RT for 60 min in a moist chamber. After rinsing in PBS, a peroxidase conjugated-mouse IgG (NOVO Castra Laboratories LTD., UK) secondary antibody was applied at RT for 30 min. The peroxidase activity in tissue sections was visualized by utilizing a variety of chromogens. Diaminobenzidine (DAB) produces reddish brown

precipitate in the sections. This was used to localize chymase. Vector SG substrate developing a blue-black reaction product, was utilize to visualize tryptase in the cell sections. Vector VIP peroxidase substrate, produce an intense purple precipitate was implied in order to recognize eosinophils. All three chromogenic systems are available as kits and purchased from Vector Laboratories, Immunkemi, Järfälla, Sweden. To confirm a positive staining, a spleen processed similar to the experimental specimens, was used. All sections were counter-stained with Light green. Sections without primary antibodies served as negative control.

To verify the results from immunohistochemical labelling, the tissues were simultaneously stained with Toluidine blue for counting mast cells and Papenheim (a combination of May-Grünwald and Giemsa) for eosinophils [17].

2.4. Conventional histological methods

In all cases, sections from the heart, both lungs, liver and kidney were stained with Haematoxylin-erythrosine-saffron and Mallory's PTAH for assessment and histological changes. In all cases in anaphylaxis and asthma death groups, sections from the brain, thyroid, adrenals and spleen were also studied.

2.5. Histomorphometry analysis of immunostaining

Fifty visual fields per each myocardial section in magnification $\times 400$ were selected randomly and all MC-TC, MC-T, MC-C and eosinophil (immunopositive stained cells) were counted by Planophotometry (DP-Soft Imaging System GmbH). The total number of immunopositive MCs and eosinophils throughout the entire heart sections were expressed as number of cell per 3mm². Each visual field per lung section in magnification $\times 400$ was randomly chosen and the numbers of the immunostained cells surrounding bronchioles in a total area of 1.5 mm² were counted. These cells were designated bronchial mast cells and eosinophils. Likewise, the equivalent areas in the same slides without bronchi were assessed and the counted-immunoreactive cells were regarded as parenchymal mast cells and eosinophils. To better evaluate the results obtained from the immunohistochemical analysis, a group of explanatory parameters were designed for each case (refer to Table 2). The sum of both MC-TC and MC-T per tissue area was considered as MCs, likewise for eosinophils. By lung MC-TC, lung MC-T and lung MC-C we considered the addition of amount of bronchial and parenchymal of these type of cells together. By lung MCs, we indicated the sum of both bronchial and parenchymal MC-TC together with MC-T and MC-C per tissue area. By cardiac MCs we mean the sum of MC-TC, MC-C and MC-T in heart tissue.

2.6. Statistical analysis

All statistics were calculated using non-parametric tests. Computations of significances between two groups were performed using the Mann–Whitney *U*-test. In comparison of several means the Kruskal–Wallis test was used. For correlation within groups, Spearman's rank correlation test was used. All calculations were performed using the statistical package Statistica, (Statsoft, Scandinavia AB). *P* < 0.05 was regarded as significant. The number of counted cells was expressed as the mean $\pm$ S.E.M. per tissue area.

3. Results

The postmortem tryptase values, the cause of anaphylaxis, the sex/age information and the anaphylactic circumstances in the nine cases of Group I are shown in Table 1. All cases in Group I was given a postmortem diagnosis of anaphylaxis after inquest. In all cases there was a history of collapse after contact with allergen. The allergen was documented for all cases except for one patient where it was not possible to identify before the agonal period of the anaphylaxis, but was accepted as being the result of anaphylaxis after the finding of a very high mast cell tryptase concentration and exclusion of other possible

Table 1
Presentation of the nine of anaphylaxis cases

Number of cases	Sex/age	Cause of anaphylaxis	Laryngeal edema	Pulmonary edema	Femoral tryptase $\mu\text{g/l}$	Specific serum IgE identified
1	F/21	Pets	Yes	Yes	NI	NI
2	M/44	Penicillin-V	Yes	Yes	28 ^a	Yes
3	M/45	Unknown	Yes	Yes	3300 ^a	NI
4	M/47	Mite	Yes	Yes	200 ^b	Yes
5	M/55	Pollen	Yes	Yes	320 ^b	Yes
6	M/50	Bee sting	Yes	Yes	180 ^b	Yes
7	M/17	Food	No	Yes	5,5 ^a	Yes
8	M/23	Penicillin-V	No	Yes	29 ^b	Yes
9	M/55	Diagnostic agent	Yes	Yes	480 ^b	No

NI, not identified.

^a β -tryptase was measured.

^b Both α - and β -tryptase were measured.

explanation for the cause of death. The autopsy findings were similar in each case of anaphylactic death (Group I) with the exception of the lack of laryngeal edema in two cases. In the histological sections a rather discrete non-specific inflammation was seen in the airways. None of the patients in this group had cardiovascular disease except in one case that had discrete myocardial fibrosis as seen in microscopically examination. The asthma deaths (Table 2) showed acute pulmonary emphysema in all cases sometimes congestion but seldom edema. Histologically a conspicuous inflammatory infiltrate featuring eosinophils and some neutrophils were always observed in the airway mucosa and bronchial walls. The cause of asthma was not specified on the hospital records of patients who died from asthma, but environmental factors seemed to be most likely the cause of death. In this group, neither α - nor β -tryptase were measured postmortem except for one case (Table 2). The asthma deaths occurred rapidly and mostly outside the hospital. The lack of certain information can be explained by external circumstances for collecting them and that the diagnosis of asthma in some cases were not made prior to death and was first diagnosed at autopsy.

Utilizing antibodies against human tryptase and chymase and a double immunohistochemical staining, we managed to distinguish all three subsets of mast cells. In order to immunostaining eosinophils, we used antibody to MBP as a

marker [18]. Mast cell-subsets differentiated and studied immunohistochemically. Fig. 1 shows the mast cell-subsets: MC-TC, MC-T, MC-C and the eosinophils in lung sections, to illustrate the differences in immunostaining between these cells. Counted eosinophils by May-Grünwald-Giemsa showed comparable result with MBP labelled cells. However many mast cell may have failed to stain with toluidine blue due to extensive degranulation. Although according to our results the number of toluidine stained MCs was underestimated by approximately 20% compared with the number of immunologically labelled cells.

Mast cell β -tryptase is a protein that is released from mast cell granules during anaphylactic and anaphylactoid reactions. α -tryptase is a similar protein that is secreted by resting mast cells [19]. No significant correlation was found with respect to the number of infiltrated mast cells or eosinophils at various tissue localizations and the level of serum tryptase. This result could be explained by the fact that the postmortem concentration of tryptase altered after a certain period of time while the numbers of infiltrated cells after death instant remain unchanged. Moreover, the tryptase level on patients 7 and 8 was under cut off value, but they were included in the anaphylaxis group due to the manner of death.

In Group I-lung sections, there were significant higher numbers of infiltrated mast cell-subsets including MC-TC,

Table 2
Presentation of 10 cases who died in acute severe bronchial asthma

Number of cases	Sex/age	Laryngeal edema	Pulmonary edema	Pulmonary emphysema	Mucous plugging
1	F/47	No	No	Yes	Yes
2	F/40	No	Yes	Yes	Yes
3	M/54	No	Yes	Yes	Yes
4	M/58	No	Yes	Yes	Yes
5	M/15	No	Yes	Yes	Yes
6	M/58	No	No	Yes	Yes
7	M/50	No	No	Yes	Yes
8	M/28	No	No	Yes	Yes
9	F/60	No	Yes	Yes	Yes
10	M/24	No	No	Yes	Yes

Concentration of femoral tryptase and the specific serum IgE was not measured in this group except in Case 5 that both α and β tryptase was measured and this was 72 $\mu\text{g/l}$.

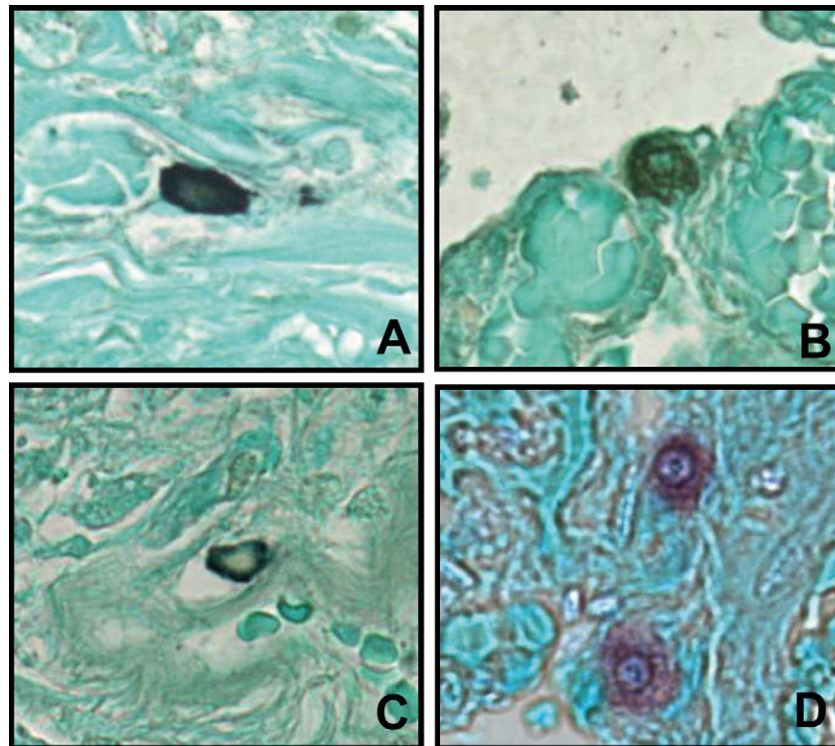


Fig. 1. Immunohistochemical staining of mast cell-subsets and eosinophil in lung tissue. (A) Immunoreactivity of anti-tryptase and anti-chymase of the same mast cell located within lung parenchymal. (B) Intracellular staining of only chymase in mast cell by utilizing anti-chymase. The cell situated within alveolar septa. (C) Mast cell immunopositive for anti-tryptase alone. (D) Immunoreactivity of eosinophil for anti-BMP located in lung parenchymal tissue. Magnification $\times 400$.

MC-T and MC-C ($P < 0.01$) at the site of bronchial smooth muscle and parenchymal tissues compared with all other groups (Table 3). There were no significant differences between the amount of infiltrated MCs in fatal asthmatic (Group II) and the control (Group IV) as shown in Table 3. The

numbers of infiltrated MCs and eosinophils surrounding bronchial smooth muscle were elevated in the lung tissues of anaphylaxis cases (Group I) as compared with parenchymal area in the same group or with Group III and IV ($P < 0.05$). The infiltration of mast cells containing only tryptase was increased

Table 3
Mean number of mast cell-subtypes and eosinophils in the lung and heart sections of study subjects

Explanatory parameters	Total area (mm ²)	Mean $\pm$ S.E.M.			
		Group I	Group II	Group III	Group IV
		N = 9	N = 10	N = 10	N = 20
Bronchial MCs	1.5	44 $\pm$ 8.7 ^a	22.3 $\pm$ 6	11.2 $\pm$ 4.6	17 $\pm$ 2.4
Parenchymal MCs	1.5	27 $\pm$ 4.4 ^a	11.7 $\pm$ 2.9	5.3 $\pm$ 2.8	16 $\pm$ 1.7
Bronchial eosinophils	1.5	37.5 $\pm$ 9.8 ^b	127.6 $\pm$ 72 ^a	9.2 $\pm$ 3.6	8 $\pm$ 1.8
Parenchymal eosinophils	1.5	27 $\pm$ 6.4 ^a	18 $\pm$ 6.5 ^b	6.9 $\pm$ 2	6 $\pm$ 1
Lung MC-TC	3	53 $\pm$ 9 ^a	27 $\pm$ 7	14.3 $\pm$ 6	27 $\pm$ 3.3
Lung MC-T	3	16 $\pm$ 2.8 ^a	6.6 $\pm$ 1.8	2.2 $\pm$ 1.1	6.3 $\pm$ 1.2
Lung MC-C	3	2 $\pm$ 0.8	0	0	0
Lung eosinophil/lung MC	3	1 $\pm$ 0.3	6.7 $\pm$ 3.8	0.31 $\pm$ 0.1	0.65 $\pm$ 0.1
Cardiac MCs	3	19 $\pm$ 1.9	14.2 $\pm$ 1.6	5.8 $\pm$ 1.5	15.3 $\pm$ 0.8
Cardiac MC-TC	3	18 $\pm$ 2	13.3 $\pm$ 2	5.7 $\pm$ 1.5	15.4 $\pm$ 0.84
Cardiac eosinophils	3	8 $\pm$ 2.3	3.8 $\pm$ 1	2.9 $\pm$ 1.4	7.1 $\pm$ 1.2
Cardiac eosinophil/cardiac MC	3	0.5 $\pm$ 0.1	0.24 $\pm$ 0.01	0.2 $\pm$ 0.1	0.47 $\pm$ 0.07

Group I: fatal anaphylaxis.

Group II: asthma death.

Group III: asthmatic patient how died from other causes.

Group IV: control group.

^a Significant differences according to Kruskal–Wallis test between four groups.

^b Significant differences between one group compared with control group according to Mann–Whitney *U*-test.

significantly in the anaphylaxis group compared with all the other groups (Table 3). In the asthmatic deaths (Group II) the number of infiltrated eosinophils surrounding bronchial smooth muscle were increased dramatically compared with infiltrated parenchymal eosinophils in the same group or with the all other groups ($P < 0.001$) while the infiltrated MCs were increased compared with control group ($P < 0.01$) as shown in Table 3. In anaphylaxis cases (Group I) there was an average of one eosinophil contra one mast cell, while in asthmatic death (Group II) the ratio increase in average of six to seven eosinophils challenging one mast cell (Table 3). Deaths in Group III that were caused by non-immunological disease agreed with the obtained results that showed a significant lower amount of infiltrated MCs and eosinophils in the lung and heart tissues (Table 3). While the MC-TC was the dominant mast cell-subset that infiltrated into the lung tissue of the all study groups, the number of the MC-TC infiltrating into the lung of anaphylaxis cases increased profoundly compared with the other groups, $P < 0.05$ (Table 3).

A distinctive result of immunohistochemical analysis was the finding of MC-C only in the lung of anaphylaxis victims ranged from 1 to 10 with a mean number of 2 ± 0.8 (mean $\pm$ S.E.M. (Table 2)). The amount of MC-C was small but the finding was unique for the cellular composition of the anaphylaxis-lung tissues. The great change of Groups I and II-lung cellular composition concerning mast cell-subsets and eosinophils was not evident in the cardiac tissues (Table 3). Only the amount of infiltrating cardiac eosinophils and MCs were higher in anaphylaxis (Group I) compared with the other groups (Table 3). The ratio between cardiac eosinophil to MC was increased due to the increasing number of infiltrated eosinophils in Group I. There was no MC-C in the heart tissue of the groups and the amount of MC-TC predominated among the cardiac MCs.

4. Discussion

It is well established that infiltration by mast cells, eosinophils and activated T-lymphocytes play a central role in anaphylactic reactions and asthmatic airway inflammation. The intriguing finding of this study was the marked differences of cellular composition of the lung between fatal anaphylaxis and asthmatic death. The anaphylactic deaths did show neither emphysema nor significant mucous bronchial secretions whereas all asthmatic deaths did. The degree of pulmonary congestion and edema was also severe in anaphylaxis. This corresponded with the histological findings and the location and number of mast cell-subsets and eosinophils in the different compartments of the lungs. The symptomatology of anaphylaxis with mucosal and parenchymal edema could be explained by activation of MCs both centrally and peripherally in the lung parenchyma. This would result mainly in a generally increased vascular permeability and in combination with a systemic vasodilation also to severe congestion. In acute asthma on the other hand the effectors cells (eosinophils and fewer MCs) were located mainly in the bronchial wall and mucosa leading to the typical symptoms of bronchial smooth muscle constriction and mucous secretion.

Amin and his co-worker showed by measuring the extracellular deposition of mast cell products that in allergic asthma the remodelling of bronchial compartments was caused by a significant accumulation of mast cells [20]. According to the results of our investigation the airways of patients who died from asthma is mainly characterized by an excess infiltration of eosinophils and less so of mast cells. Likewise our results suggest that in the prophase of asthma mast cells mediators may promote early bronchial constriction and recruitment of lymphocytes and eosinophils and thus contribute to the disordered airway physiology and remodelling. While in the late phase of asthmatic reactions mast cells do not contribute so much to the pathophysiology of this disease and airway dysfunction in asthma correlate with the release of the potent inflammatory mediators of infiltrated eosinophils.

The different physiological functions of the mast cell-subtypes that we have studied are not completely known. Significant increases of all three subtypes were only seen in the anaphylactic deaths. This increase seemed to be proportional, i.e. all the subtypes were increased by the same factor (2–2.5) compared with the controls. MC-TCs (formerly called connective tissue mast cells) are mainly composed of histamine, heparin, tryptase, chymase, cathepsin G and carboxypeptidase, which are preformed and stored in granules. MC-T (formerly mucosal mast cells) lack or contain only small amounts of chymase, carboxypeptidase and cathepsin G [21]. MC-Cs on the other hand lack tryptase but have not been further characterized and their existence is not yet fully accepted. The proportions of the other constituents in these subsets are not known. Furthermore, mast cells have the capacity to synthesis numerous lipid mediators such as leukotrienes, prostaglandins, chemokines and cytokines. Studies have been performed on the production of lipid mediators from different tissues but not from the mast cell-subsets per se [22].

We found that the mast cells in the heart were increased in anaphylactic deaths, but not in asthma. This is consistent with the view that anaphylaxis is a systemic disorder of the vasculature and that asthma is a local disease of the bronchi. The cardiac mast cells are of the MC-TC type and are situated in the interstitium and in the adventitia of larger coronary vessels. It has been debated whether the deterioration of heart function in anaphylaxis is influenced by local mast cell activation or only by mast cell products emanating from the lung [23]. Our results corroborates the former view. It would also be of interest to compare the mast cell content of extra-cardiac blood vessels in anaphylaxis and controls. To our knowledge this has not been done so far, but experimental studies previously have made clear that both the aorta and peripheral vessels are influenced by mast cell mediators [24]. The mechanisms underlying the augmented infiltration of MCs into the tissues is not fully understood. One can speculate that MCs tend to be located perivascularly to respond to stimulus as well as allergens. MC expresses the high-affinity receptor for IgE. Cross linking of IgE by allergens or degradation products of allergen leads to activation of the pre-existing MCs and consequently release of various mediators. MCs are capable of secreting a plethora of cytokines and chemokines that can

activate lymphocytes and macrophages. These include the cytokines (TNF α , IL1 β , IL4, IL5, IL8 and IL13 (13), lipid mediators and histamine, which can have profound effects on vascular endothelium, including the alteration of vascular permeability and adhesiveness. This can allow other circulating MCs and inflammatory cells to adhere and emigrate into cardiac tissues. In addition, it is possible that allergic subjects have increased numbers of pre-existing MCs and eosinophils in the myocardium due to previous or repeated allergic reactions.

In conclusion we have shown that the cellular infiltrate in anaphylaxis and acute asthma differs significantly and for the first time that the number of mast cells in the lung and the heart are significantly increased in anaphylactic reaction compared to controls. The mast cell-subsets are increased to an equal degree and we have demonstrated that the third type of mast cell MC-C is only found in the lungs in anaphylactic deaths. The increased number of mast cells seems to be a pre-condition for a severe anaphylactic reaction. In another study we have shown the same increase in lung mast cells in drug abusers dying suddenly after injection of narcotics (unpublished). The practical consequence of our study will be that it is now possible to confirm a suspicion of anaphylaxis death not only by measurements of serum mast cell tryptase, but also by immunohistochemical methods. The meaning of our results, however, has to be further investigated by other experimental methods.

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