Mast Cell Tryptase Levels in Gut Mucosa in Patients with Gastrointestinal Symptoms Caused by Food Allergy

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Key Words

Tryptase · Gastrointestinal food allergy · Mast cells

Abstract

Background and Aims: Mast cells, which are important effector cells in food allergy, require a special histologic treatment for quantification in endoscopic gastrointestinal samples. The objective of this study was to investigate whether mast cell tryptase (T), a typical mast cell-associated marker, may help to detect patients with food allergy. **Methods:** Mast cell T was investigated from 289 colorectal samples of 73 controls, 302 samples from 43 patients with food allergy and gastrointestinal symptoms, and 72 samples from 12 patients with partial or complete remission of allergic symptoms. Endoscopically taken samples were immediately put into liquid nitrogen, mechanically homogenized by a micro-dismembrator with three homogenization steps and tissue T content (ng T/mg wet weight) was measured by fluoroenzyme immunoassay. **Results:** Tissue T levels from the lower gastrointestinal tract were significantly elevated ($p < 0.0001$) in patients with manifest gastrointestinal allergy (median: 55.7, range: 9.3–525.0) compared with controls (median: 33.5, range: 8.0–154.6). A subgroup of 12 patients with remission of allergy showed markedly decreased symptom scores and

mucosal T levels after more than 1 year of antiallergic therapy (pretreatment median: 54.1, range: 37.0–525.0 and post-treatment median: 28.4, range: 19.8–69.1; $p = 0.01$). **Conclusions:** High T levels in the gut of food-allergic patients support the role of stimulated mast cells or an increased mast cell number.

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Introduction

The term ‘food allergy’ includes different types of immunologically mediated food hypersensitivity, which explains the occurrence of various clinical pictures and involvement of several shock organs (skin, intestine, cardiopulmonary system, etc.) [1–3]. One possible pathway represents the degranulation of mast cells and the consecutive release of mediators and proteases followed by an enhancement of the intestinal mucosal permeability resulting in an increased influx of potential allergens and harmful microbes into the intestinal tissue [4–6]. Furthermore, IgE-stimulated mast cells trigger enteric nerves resulting in pain and diarrhea, two of the leading symptoms in food allergy [7]. Of the great variety of mast cell mediators, tryptase (T) represents a nearly unique mast

cell protease. In contrast to histamine, which may stem from several sources (mast cells, basophils, enterochromaffin cells, etc. [8]) and which is only a mercurial molecule due to its short half-life (e.g. 2–3 min), T is mainly found in mast cells and, to a much lesser extent, in basophils [9–11]. In the circulation, its half-life amounts to approximately 120–180 min [8, 9, 11, 12]. These advantages of T prompted us to investigate whether the detection of mast cell T in gut mucosa can help to detect patients with gastrointestinal allergy.

Materials and Methods

Patient Groups

Forty-three patients with proven food allergy were included in the study (mean age 41.2 ± 15 years, 28 female, 15 male). Allergy diagnosis included history, skin-prick tests (Bencard, Smith Kline Beecham, Germany), and total and food-specific serum IgE (CAP FEIA; Pharmacia CAP system, Sweden). Food allergy was confirmed in each patient by double-blind placebo-controlled food challenge (DBPCFC). All 43 patients presented with gastrointestinal symptoms (diarrhea or loose stools 100%, abdominal pain 60%, bloating 56%, nausea or vomiting 28%), whereas asthma and dyspnea (23%), skin pruritus or flush (41%), tachycardia (28%), rhinitis (11%) or migraine (11%) occurred less frequently.

An extensive work-up had to be performed until patients were subjected to DBPCFC. The work-up included serology, stool examinations, H₂-breath tests (lactose, fructose, sorbitol), fecal elastase 1, upper and lower gastrointestinal endoscopy, small bowel enteroclysis, psychosomatic exploration, allergological testing including measurement of urinary methylhistamine and sometimes of 5-hydroxyindolic acid [13–16]. During colonoscopy mucosal samples were taken from the terminal ileum and all parts of the colon for histology to exclude other inflammatory disorders of the gut. For biochemical mediator detection (tissue tryptase, T_T), samples were taken from the terminal ileum, ascending colon, transverse colon and recto sigmoid. In 4/43 allergic patients (9.3%) a small colorectal adenoma (<5 mm) was found, while other diseases could be excluded.

At the time of colonoscopy no patient had been under antiallergic, immunosuppressive or steroid treatment for at least 2 weeks. Patients were prepared for colonoscopy using a commercial polyethyleneglycol solution. To facilitate colonoscopy, midazolam/diazepam and meperidine were used at a dose of 2.5–10 mg and 25–150 mg, respectively, in 38/43 patients (88.3%) with food allergy and in 48/73 controls (65.7%) [17].

Standardized DBPCFC was performed in 43/43 patients (100%) who were diagnosed as allergic enteropathy, and were hospitalized [9, 16, 18, 19]. Food allergy was only diagnosed when food-specific sensitization was detectable by positive skin-prick test, serum IgE ($\geq$ class II) or proof of intestinal IgE by endoscopically guided segmental lavage [2, 16, 20] in conjunction with a reproducible clinical adverse reaction to the food antigen(s) applied. Food antigens identified were cereals, soy, nuts, milk, beef, pork, egg and pollen-cross reactive allergens (apple, celery, etc.). All patients with confirmed allergy and controls were classified as atopics or nonatopics if they had a positive or negative history

for allergic rhinitis or conjunctivitis, asthma bronchiale or atopic eczema, respectively [14, 21].

The 73 controls (mean age 43.4 ± 27.1 years, 42 female, 31 male) were not suffering from either inflammatory or allergic processes in the gut. Fifteen of these 73 patients (20.5%) were atopics but did not complain of gastrointestinal symptoms consistent with food allergy. All controls underwent colonoscopy for anemia work-up, screening for colorectal neoplasia, evaluation of constipation, abdominal pain and perianal bleeding. With the exception of hemorrhoids (13/73 patients, 17.8%), anemia due to intestinal angiodysplasia (11/73 patients, 15.0%), diverticulosis (11/73 patients, 15.0%) and colorectal adenoma (8/73 patients, 10.9%), neither endoscopy nor histology revealed any signs of gastrointestinal disease (30/73 patients, 41.0%). Food hypersensitivity was excluded by allergological history and, if necessary, by skin-prick tests and total or antigen-specific serum-IgE.

Biopsy Sampling and Tissue Homogenization

During colonoscopy (EC 3800F, EC 3801F2 Pentax, Hamburg, Germany) 4–7 samples were taken from each patient for T detection after having obtained informed consent. The study was approved by the Ethics Committee (No. 331) of the University of Erlangen-Nuremberg.

For investigation of the total T_T content, biopsies were immediately put into liquid nitrogen (-72°C) in the endoscopic unit. In the lab, biopsy wet weights (w.w.) were measured by the use of a microbalance (Sartorius, Germany). Mechanical homogenization of the biopsy was performed using a micro-dismembrator (Braun-Biotech, Germany) within 60 min of sample collection [1, 15, 17]. One tissue sample was homogenized by three homogenization steps using: (1) 1,500 μl aqua bidestillata (1,500 rpm, 5 min), (2) 1,500 μl aqua bidestillata (1,500 rpm, 3 min) and (3) 1,500 μl modified Hanks' medium supplemented with albumin, PIPES, HEPES buffer and fetal calf serum. Homogenization steps 2 and 3 were performed to wash the Teflon homogenization tube (from 1), since former studies have shown a mediator to be binding to the tubes [7]. All homogenization fluids were separately frozen at -20°C until T_T measurement was performed within 10 days of sample collection. The total T_T content from one sample resulted from addition of the mediator amounts detected in 1–3 and was expressed in ng T_T/mg w.w.

T standards with concentrations of 0, 10 and 50 $\mu\text{g/l}$ T were used to evaluate the homogenization procedure and T measurement. Using this homogenization system resulted in the following recovery rates (each 12 experiments): for the 0 $\mu\text{g/l}$ standard $0.1 \pm 1.1\%$ were found; for the 10 $\mu\text{g/l}$ standard $92.0 \pm 14.8\%$ were detected and for the 50 $\mu\text{g/l}$ standard $78.9 \pm 31.6\%$ were recovered.

During the tissue homogenization procedure $80.8 \pm 18\%$ of the total T_T content from one biopsy were obtained by the first homogenization step 1, whereas steps 2 and 3 retrieved $14.3 \pm 14\%$ or $5.5 \pm 7\%$, respectively ($n = 409$ samples; no difference between food allergic patients and controls).

T Measurement, Assay Characteristics and Statistical Analysis

T_T detection was made by a specific fluoroenzyme immunoassay (Tryptase Unicap, Pharmacia) according to the manufacturer's instructions [5]. Intra- and inter-assay variations were 12 and 12.8%.

Fig. 1. Distribution of mast cell T levels in normal human gut mucosa and patients with allergic enteropathy. The plot contains all T values from the lower gastrointestinal tract of 73 controls and 43 patients with allergic enteropathy (at the time when presenting with manifest allergic disease). Each symbol represents the average T value of all biopsies ($n = 4-7$) taken from individual patients. The grey rectangle indicates the normal range of gut mucosal T levels and its diagnostic level of 45 ng T/mg w.w. Horizontal lines within each group indicate the median and the numbers given above and below the diagnostic level represent the number of patients exceeding or falling below this level with their corresponding T levels. Diagnostic sensitivity of T detection to identify allergic enteropathy was thus calculated to be 72.1%, specificity 72.6%, positive predictive value 60.7% and negative predictive value 81.5%. * $p < 0.0001$ (control group vs. allergic enteropathy).

The average T_T content of each patient was calculated as the mean from all samples ($n = 4-7$) obtained from one person. This average T_T value was then used for final statistics. The regional variation of T_T levels from all biopsies taken from the lower gastrointestinal tract from one person was on average $26.1 \pm 12\%$ in allergic enteropathy and $21.1 \pm 12.3\%$ in controls.

For statistics, comparisons were made between food allergy and controls using median, range and the U test (Wilcoxon, Mann-Whitney, two-sided). Sensitivity, specificity, positive and negative predictive values of gut mucosal T_T detection were calculated for identification of individuals with allergic enteropathy using a two-by-two contingency table [18].

Results

Frequency of Atopy

In the group with confirmed food allergy 20/43 patients (46.5%) were atopics, while the remaining patients (21/43, 48.8%) were nonatopics. 2/43 patients (4.6%) could not be classified because of uncertainties. Among controls without food allergy, 15/73 patients (20.5%) were atopics, while 58/73 patients (79.4%) did not show any signs of atopic disorders.

Mean T Content of the Lower Gastrointestinal Tract

Gut mucosal T_T levels (all values in ng T_T /mg w.w.) were significantly elevated ($p < 0.0001$; fig. 1) in patients with food allergy and gastrointestinal symptoms (medi-

an: 55.7, range: 9.3–525, $n = 302$ biopsies) compared with controls (median: 33.7, range: 8.0–154.6, $n = 289$ biopsies).

Analysis of the patients with food allergy according to atopics or nonatopics revealed that the 20 patients with atopic disorders had a median T_T level of 50.6 (22.7–525.0) and the remaining 21 nonatopics had a median T_T level of 72.6 (9.3–143.0; $p = 0.2$; fig. 2), and both were significantly elevated compared with the T_T levels from atopic (median: 33.5, range: 21.5–106.2; $p = 0.03$) and nonatopic controls (median: 32.7, range: 8.0–154.6; $p < 0.0001$). The presence of atopy alone was not accompanied with significantly elevated gut mucosal T_T content in allergic enteropathy or in controls (fig. 2).

Correlation of Gut Mucosal T_T Levels with Disease Activity (Symptom Score)

In 12 voluntary patients with allergic enteropathy who agreed to undergo recolonoscopy after a median follow-up of 17 months with antiallergic therapy (antigen avoidance, disodium cromoglycate, oligopeptide diet) and partial (1/12 patients, 8.3%) or complete remission (11/12 patients 91.6%) of symptoms, T_T levels were again measured and correlated with the clinically observed symptom score. As shown in table 1, T_T levels declined after allergy treatment in all 11 patients with complete remission, out of a total of 12 patients. However, the patient with a partial response to the antiallergic diet showed a further increase

Fig. 2. Differentiation of gut mucosal T content of atopic and nonatopic controls and patients with allergic enteropathy. The plot shows the T values from the lower gastrointestinal tract of 15 atopic and 58 nonatopic controls as well as of 20 atopic and 21 nonatopic patients with confirmed food allergy. Each symbol represents the average T value of all biopsies ($n = 4-7$) taken from individual patients. The grey rectangle indicates the normal range of gut mucosal T levels and its diagnostic level of 45 ng T/mg w.w. Horizontal lines within each group indicate the median. Allergic enteropathy atopics vs. atopics of controls, $p = 0.03$; allergic enteropathy nonatopics vs. nonatopics of controls, $p < 0.0001$.

in the mucosal T_T content, possibly suggesting that antigen avoidance was not complete. Pre-therapeutic T_T levels of these 12 patients (median: 54.1, range: 37.0–525.0) and the symptom score (median: 6.3, range: 2.9–12.8 points) were clearly different from post-therapeutic values of T (median: 28.4, range: 19.8–69.1; $p = 0.01$) and the symptom score (median: 2.2, range: 0–5.8). Interestingly, the T_T content was found to moderately correlate with disease activity ($r = 0.58$, $p = 0.005$). When comparing post-therapeutic T_T levels of these 12 patients with remission of food allergy (median: 28.4, range: 19.8–69.1) with the whole control group (median: 33.5, range: 8–154.6) no statistical difference could be detected ($p = 1.0$).

Identification of Gastrointestinal Allergy by Gut Mucosal T_T Measurement

The distribution of all T_T levels obtained in normal colorectal mucosa and patients with food allergy is given in figure 1. When assuming 45 ng T/mg w.w. as a diagnostic level, T_T detection yielded a sensitivity of 72.1%, specificity of 72.6%, positive predictive value of 60.8% and a negative predictive value of 81.5% (fig. 1).

Discussion

Food allergy can be caused by various pathomechanisms and lead to a plethora of clinical symptoms, e.g. abdominal cramps, bloating, diarrhea, flush, rhinitis,

Table 1. Pre- and post-therapeutic levels of gut mucosal T content in 12 patients with confirmed food allergy after partial or complete remission of allergy

Patient No.	Pre-therapeutic gut mucosal T content ng T/mg w.w.	Post-therapeutic gut mucosal T content ng T/mg w.w.	Follow-up with rebiopsy time months
1	37.7	69.1	12
2	37.0	19.8	18
3	65.7	20.3	12
4	41.5	25.4	16
5	525.0	28.4	21
6	55.7	47.5	20
7	37.8	19.3	24
8	69.9	55.7	21
9	109.2	46.5	18
10	72.6	50.9	12
11	52.5	28.5	16
12	40.6	24.3	12
Total patients n = 12	Median 54.1 (37.0–525.0)	Median 28.4* (19.3–69.1)	Median 17 months (12–24 months)

Gut mucosal T content was determined in a subgroup of 12 patients with allergic enteropathy before and at least 12 months after antiallergic treatment. Remission of allergic symptoms was evaluated by a clinical symptom score. Patient 1 was found to have only partial remission of allergy, while in all other patients a complete remission of allergic enteropathy was accompanied with a significant reduction in T levels.

* Pre- and post-therapeutic medians of T levels in each group were significantly different, $p = 0.01$.

anemia, etc. [1, 14, 22–24]. One possible pathway, the neutral serine protease T_T from endoscopically taken gut mucosal samples was analyzed in this study.

Analysis of the T_T content revealed that atopic or non-atopic patients with manifest food allergy had on average significantly elevated T_T levels than the corresponding groups of control patients. Although the medians of both groups differ significantly (fig. 1), T_T values in allergic individuals show a wide range of distribution, indicating that intestinal mucosal mast cells are distributed rather in a patchy way than a regular one [8, 17, 20, 25, 26]. This variation of mast cell density or T_T content may well indicate the existence of several clinically distinct and local forms of food allergy, which have been described as allergic proctitis, allergic enterocolitis or allergic ileitis [14, 21, 27, 28]. According to the location of the main mast cell infiltrates, IgE cross-linking by food antigens on mast cells may thus induce a plethora of various gastrointestinal complaints, ranging from simple or excruciating bloating to diarrhea or severe malabsorption.

To overcome the known problem of intra- and inter-individual variations of the mediator content, 2–3 biopsies from each segment of the gut should be taken in the future for biochemical analysis to adequately recognize areas with increased T_T levels. When calculating an average T_T level from all samples of the lower gastrointestinal tract from each patient, 72% of patients with gastrointestinal food allergy were identified by T_T levels above 45 ng T/mg w.w. (sensitivity), whereas less than 30% of controls exceeded this level (specificity 72.6%), providing a positive predictive value of approximately 61% and a negative predictive value of 81.5%. Patients with T_T levels below 45 ng/mg w.w. in the lower gastrointestinal tract might have: (1) a non-IgE-mediated (e.g. type III or IV) allergy [13, 21, 29], (2) a lower disease activity [1, 14, 20–22, 24–28, 30] or (3) the allergic process might be located in the upper gastrointestinal tract.

As shown in 12 patients with remission of food allergy, T_T levels decline significantly to a normal range when the offending antigen is effectively eliminated or mast cell reactivity additionally inhibited by disodium cromoglycate. Gut mucosal T_T levels appear thus to be one factor influencing clinical allergy activity as evidenced by the use of a standardized symptom score.

In contrast to this finding, serum T has rarely been shown to rise after food-induced allergic reactions [29, 31], except in very severe reactions or anaphylaxis. This discrepancy may be explained by the great tissue-binding capacity of T to negatively charged macromolecules (heparin, chondroitin sulfate E) [9, 11]. Once released from

activated effector cells, T rapidly becomes a tissue-bound enzyme which exerts its proteolytic activity within the tissue (e.g. substrates: collagenase, complement factors, proteinase-activated receptor PAR₂, type IV collagen). This binding of T to tissue constituents and its tissue-predominant action may well explain why detection of the T_T content from endoscopic samples enables the identification of more than two-thirds of patients with food allergy and gastrointestinal symptoms, and correlates better with disease activity than serum T determinations [29, 31]. In addition, mast cells form an important constituent of the immune cell infiltrate within the gastrointestinal tract and interact with other cell types like eosinophils, intestinal lymphocytes, fibroblasts and smooth muscle cells by their peculiar mediator secretion. T is known to activate eosinophils along with TNF- α and other mast cell products, while activated eosinophils express proteinase-activated receptor 2 receptors and, in turn, continue to activate mast cells via basic protein secretion. This cellular crosstalk via soluble and physical signals by mast cells is not only confined to intestinal eosinophils, but also to other tissue constituents and presents the pathophysiological basis for an upregulated allergic effector cell unit, unless the intestine is confronted with causative food antigens. T is further involved, at least in part, in smooth muscle activation and fibroblast stimulation, which might indicate that long-term activation of intestinal mast cells may also be associated with tissue remodeling and functional disturbances in patients with food allergy [6, 9, 12, 31, 32]. As we have shown that gut mucosal biopsies of patients with gastrointestinal symptoms caused by food allergy contain high amounts of T, it appears likely that the various mentioned effects of T contribute to the clinical phenotype of gastrointestinal allergy with impaired barrier function, proteinase-activated receptor 2 activation, inducing visceral and somatic hypersensitivity via smooth muscle or fibroblast activation, and increasing neuronal excitability as has been shown recently by mast cell supernatants containing either histamine or T [9].

Limitations of the Study

In this study, the mast cell numbers were not evaluated. This removed the possibility of differentiating between an increase in mast cell numbers (e.g. by migration) and an increase in T synthesis. It also meant that we were unable to assess immunohistochemically which mast cell subtype, T-positive mucosal mast cells and/or

T- and chymase-positive mast cells from lamina propria or deeper layers, expands in gastrointestinal allergy, and whether a change in the pattern of the mast cell subtype occurs.

In addition, basophils could represent an alternative source of T in the gut, since they may increase in allergic patients, contain small amounts of T and also be recruited to the sites of intestinal allergic inflammation [10, 21,

30]. Since basophils account for less than 1% of the overall T levels, we did not specially look for basophils as a source of T within gastrointestinal tissue samples [30].

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