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 microdismembrator 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 posttreatment 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.

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 dyspepsia (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 enteroclysma, psychosomatic exploration, allergological testing (food-specific sensitization was detectable by positive skin-prick test, serum IgE (CAP FEIA), H2-breath tests (lactose, fructose, sorbitol), fecal elastase 1, upper and lower gastrointestinal endoscopy, small bowel enteroclysma, psychosomatic exploration, allergological testing including measurement of urinary methyhistamine 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 polyethyleenglycol 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 (≥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-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 hypersensitiv-ity 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-Mbiotech, Germany) within 60 min of sample collection [1, 15, 17]. One tissue sample was homogenized by three homogenization steps using: (1) 1,500 g/l standard 78.9 g/l T were expressed in ng T-T/mg w.w.

T standards with concentrations of 0, 10 and 50 μg/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 μg/l standard 0.1 ± 1.1% were found; for the 10 μg/l standard 92.0 ± 14.8% were detected and for the 50 μg/l standard 78.9 ± 31.6% were recovered.

During the tissue homogenization procedure 80.8 ± 18% of the total T-T content from one biopsy was obtained by the first homogenization step 1, whereas steps 2 and 3 retrieved 14.3 ± 14% or 5.5 ± 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%.
The average T T content of each patient was calculated as the mean from all samples (n = 4–7) taken from individual patients. 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 ± 12% in allergic enteropathy and 21.1 ± 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 atotics, while the remaining patients (21/43, 48.8%) were nonatotics. 2/43 patients (4.6%) could not be classified because of uncertainties. Among controls without food allergy, 15/73 patients (20.5%) were atotics, while 58/73 patients (79.4%) did not show any signs of atopic disorders.

Mean T 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 (median: 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 atotics or nonatotics 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 nonatotics 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.
in the mucosal T content, possibly suggesting that antigen avoidance was not complete. Pre-therapeutic 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 content was found to moderately correlate with disease activity (r = 0.58, p = 0.005). When comparing post-therapeutic 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 Measurement**

The distribution of all 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 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** | **Post-therapeutic** | **Follow-up with rebiopsy time** |
|                 | gut mucosal T content | gut mucosal T content | 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               |

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<th><strong>Total patients n = 12</strong></th>
<th><strong>Median 54.1</strong></th>
<th><strong>Median 28.4</strong>*</th>
<th><strong>Median 17 months (12–24 months)</strong></th>
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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 exacerbating 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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References