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Lawrence B. Schwartz, M.D., Ph.D.  
*Virginia Commonwealth University, lschwartz@vcu.edu*

Dean D. Metcalfe, M.D.  
*National Institutes of Health*

Jeffrey S. Miller, M.D.  
*University of Minnesota*

Harry Earl, M.D.  
*University of Texas Health Science Center at Dallas*

Timothy Sullivan, M.D.  
*University of Texas Health Science Center at Dallas*

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## TRYPTASE LEVELS AS AN INDICATOR OF MAST-CELL ACTIVATION IN SYSTEMIC ANAPHYLAXIS AND MASTOCYTOSIS

LAWRENCE B. SCHWARTZ, M.D., PH.D., DEAN D. METCALFE, M.D., JEFFREY S. MILLER, M.D., HARRY EARL, M.D., AND TIMOTHY SULLIVAN, M.D.

**Abstract** Better methods are needed to assess mast-cell activation *in vivo* and to distinguish the activation of mast cells from that of basophils. Tryptase, a neutral protease selectively concentrated in the secretory granules of human mast cells (but not basophils), is released by mast cells together with histamine and serves as a marker of mast-cell activation.

In 17 patients with systemic mastocytosis, concentrations of tryptase in plasma were linearly related to those of histamine ( $P < 0.01$ ). Eleven of the 17 patients had tryptase levels of 4 to 88 ng per milliliter, indicating ongoing mast-cell activation. In each of six patients who experienced corresponding anaphylactic reactions after penicil-

lin, aspirin, or melon ingestion, a wasp sting, exercise, or antilymphocyte globulin injection, tryptase levels in serum ranged from 9 to 75 ng per milliliter, indicating mast-cell activation during each of these events. In contrast, serum tryptase levels were less than 5 ng per milliliter in all patients presenting with myocardial disease ( $n = 8$ , 6 with hypotension) or sepsis ( $n = 6$ , 3 with hypotension) and in the controls ( $n = 20$ ). One patient had a myocardial infarction after anaphylaxis in response to a wasp sting and an elevated tryptase level of 25 ng per milliliter. Thus, the plasma or serum tryptase level is a diagnostic correlate of mast-cell-related events. (*N Engl J Med* 1987; 316: 1622-6.)

**A**CTIVATED human mast cells secrete preformed, granule-derived mediators, including histamine, proteoglycans, and the neutral proteases called tryptase and chymase, along with newly generated mediators, including prostaglandin D<sub>2</sub>, leukotriene C<sub>4</sub>, and platelet-activating factor.<sup>1</sup> These mediators are potentially useful as clinical markers of mast-cell involvement. Plasma histamine, for example, reflects the activation of mast cells, basophils, or both types of cell during anaphylaxis,<sup>2,3</sup> as well as the high numbers of mast cells present in subjects with systemic mastocytosis.<sup>4-7</sup> However, histamine is rapidly removed from the circulation. Plasma N<sup>7</sup>-methyl histamine,<sup>8</sup> a histamine metabolite, appears to have a longer half-life in the circulation than histamine, but its measurement is technically difficult, and its presence, like that of histamine, does not indicate whether mast cells or basophils are involved. In persons with allergic asthma, for example, it is uncertain whether the rise in plasma histamine after exercise-induced bronchoconstriction is derived from circulating basophils or pulmonary mast cells.<sup>9</sup> Even during systemic anaphylaxis, the involvement of tissue mast cells has not been directly assessed.

Increased levels of a prostaglandin D<sub>2</sub> metabolite in the urine of patients with mastocytosis<sup>10</sup> and of prostaglandin D<sub>2</sub> in bronchoalveolar lavage fluid after antigen challenge<sup>11</sup> have been reported. However, measurement of prostaglandin D<sub>2</sub> or its metabolites in biologic fluids by mass fragmentography is technically difficult, and existing immunoassays lack sufficient specificity. Prostaglandin D<sub>2</sub> is also produced by platelets<sup>12</sup> and alveolar macrophages,<sup>13</sup> making identification of the cell of origin difficult when prosta-

glandin D<sub>2</sub> or its derivatives are detected in complex biologic fluids.

Assays with adequate sensitivity and specificity are not yet available for mast-cell proteoglycans *in vivo*. Other mast-cell mediators, such as leukotriene C<sub>4</sub> and platelet-activating factor, are produced by numerous other types of cells and therefore lack specificity. There is clearly a need for alternative methods to assess *in vivo* mast-cell activation specifically.

An immunoassay was recently developed to measure tryptase,<sup>14</sup> permitting the evaluation of this enzyme as a clinically useful marker of the release of mast-cell granules. Tryptase, a neutral protease, is known to be the dominant protein component of the secretory granules of each of the two types of human mast cells thus far defined: T mast cells, which contain tryptase (10 pg per lung T mast cell) but not chymase and predominate in the lung (particularly in alveoli) and intestinal mucosa, and TC mast cells, which contain both neutral proteases (35 pg of tryptase and 4.5 pg of chymase per skin TC mast cell) and predominate in the skin and intestinal submucosa.<sup>15,16</sup> Tryptase is present in all mast cells detected by metachromasia in skin, lung, and bowel tissue and in the skin lesions of subjects with systemic mastocytosis.<sup>17,18</sup> Although tryptase is present in relatively small amounts in human basophils (0.04 pg per basophil),<sup>19</sup> it has not been detected at all in other types of cells in the peripheral blood, lung, skin, and bowel.<sup>15-20</sup> Its localization to secretory granules was initially indicated by its release together with histamine from immunologically activated mast cells that had been dispersed from human lung tissue.<sup>21</sup> Tryptase and histamine also appear in skin chamber fluid overlying sites of cutaneous challenge with allergens in sensitive human subjects<sup>22</sup> and in nasal lavage fluid after challenge with allergens but not histamine.<sup>23</sup> Tryptase is stored and released as an active tetramer of 134,000 Mr that is presumably bound to the heparin or chondroitin sulfate proteoglycans that are also present in the secretory granules of mast cells.<sup>24-26</sup> The resultant macromolecular prote-

From the Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond; the Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.; and the Department of Internal Medicine, University of Texas Health Science Center at Dallas. Address reprint requests to Dr. Schwartz at Medical College of Virginia, Box 263, MCV Station, Richmond, VA 23298.

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ase–proteoglycan complex stabilizes tryptase in its active tetrameric form and may in part limit its diffusion from tissue sites of release.<sup>26</sup> The separation of tryptase from heparin permits the dissociation of the tryptase subunits from one another to form inactive monomeric subunits. Tryptase is not susceptible to tryptic or chymotryptic inhibitors normally present in plasma<sup>21,25,26</sup> and urine.<sup>27</sup>

This study uses a sandwich enzyme-linked immunosorbent assay capable of measuring both active tetrameric and inactive monomeric tryptase in plasma, serum, and experimental buffers.<sup>14,22,26</sup> Substantial levels of tryptase were found in the serum of patients undergoing clinically defined anaphylactic events, as well as in the plasma of those with systemic mastocytosis, indicating mast-cell involvement in both conditions.

## METHODS

### Subjects

Serum samples of 0.5 to 1 ml from subjects presenting with clinical evidence of anaphylaxis or hypotension associated with sepsis, myocardial infarction, or myocarditis were either taken retrospectively from serum samples collected at the time of admission or obtained after admission. Two of the patients with sepsis had end-stage renal disease and were on hemodialysis regimens. Five of six patients with sepsis and four of nine with myocardial disease were hypotensive, several requiring vasopressors. Control samples of serum were taken from 16 consecutive admission blood samples of different patients. EDTA-anticoagulated plasma was obtained from 17 patients with systemic mastocytosis as described by Meggs et al.<sup>4</sup> All the patients with mastocytosis had urticaria pigmentosa and involvement of one or more internal organs as indicated by the analysis of biopsy material, radiographic procedures, or bone and liver–spleen scans. Serum samples from six healthy volunteers were also assessed for tryptase. Plasma or serum samples were stored at temperatures of  $-20^{\circ}\text{C}$  or lower, at which tryptase immunoreactivity is stable.

The clinical characteristics of the patients with anaphylactoid reactions are summarized in Table 1 and below. In each case (except that of Patient 5), initial blood samples were drawn from one to four hours after the precipitating event. Patient 1 came to the emergency room after ingesting one tablet of penicillin V potassium. Patient 2 came to the emergency room with anaphylaxis after a wasp sting and subsequently had an inferior myocardial infarction. Patient 3 came to the emergency room after losing consciousness while exercising during the mountain cedar pollen season. Patient 4 came to the emergency room 120 minutes after eating honeydew melon and 100 minutes after the onset of the symptoms indicated in Table 1, along with obtundation, and was admitted to the coronary care unit for presumed primary myocardial disease, which was ruled out. Patient 5 had bronchospasm during desensitization to horse antilymphocyte globulin, which was to be used to suppress the rejection of a transplanted kidney. Samples were taken before and during the desensitization procedure. Patient 6 had symptoms within one hour of ingesting two adult-sized aspirin tablets and arrived at the emergency room about one hour later. All six patients recovered without sequelae.

### Histamine and Tryptase Assays

Histamine levels in plasma were determined by the radioenzyme technique,<sup>28</sup> essentially as modified by Rauls et al.<sup>29</sup> One additional modification was the use of an ultrafiltrate (Centricon-10 Microconcentrators, Amicon, Danvers, Mass.) of each plasma sample rather than untreated plasma. This step lowered background radioactivity. The mean ( $\pm$ SE) histamine level in five normal subjects as measured by this technique was  $0.12 \pm 0.02$  ng per milliliter.

Table 1. Clinical Presentation of Patients with Anaphylactic Reactions.

PATIENT No.	PRECIPITATING FACTOR	HYPOTENSION*	BRONCHOSPASM	URTICARIA ERYTHEMA	OTHER INFORMATION†
1	Penicillin V potassium tablet	+	+	+	Positive RAST for penicillin
2	Wasp sting	+	+	–	Positive RAST for wasp venom; subsequent inferior myocardial infarction
3	Exercise, mountain cedar pollen season	$\pm$	+	+	Positive skin test for mountain cedar pollen
4	Honeydew melon ingestion	$\pm$	+	+	Chest pain
5	Intradermal anti-lymphocyte globulin	–	+	–	Desensitization protocol
6	Aspirin ingestion	–	+	+	

\*The symbol  $\pm$  denotes symptoms consistent with hypotension before arrival at the emergency room, where the blood pressure was found to be normal.

†RAST denotes radioallergosorbent test.

Tryptase was measured by a sandwich enzyme-linked immunosorbent assay essentially as described previously.<sup>14</sup> Briefly, a monoclonal murine IgG2B, kappa antitryptase antibody was adsorbed to microtiter plates. Standards of purified tryptase (0.1 to 5 ng) or samples (up to 40  $\mu$ l of serum or plasma) were mixed with polyclonal goat IgG antitryptase and added to the coated microtiter wells. Alkaline phosphatase–conjugated swine antigoat IgG and *p*-nitrophenylphosphate were then added sequentially to complete the assay.

## RESULTS

The levels of tryptase in plasma from patients with systemic mastocytosis and in serum from patients with anaphylactoid reactions, myocardial infarction, myocarditis, or sepsis and from controls are shown in Figure 1. Among control patients as well as among those with sepsis or cardiac disease alone (regardless of the presence of renal failure or shock), the levels of tryptase were undetectable in 25 of 31 subjects (less than 2.5 ng per milliliter) and less than 5 ng per milliliter in 30 of 31 subjects. Tryptase levels in four healthy volunteers were undetectable (data not shown). In contrast, patients with suspected anaphylaxis had serum tryptase levels ranging from 9 to 75 ng per milliliter. In one subject (Patient 2), indicated in Figure 1 by asterisks, a myocardial infarction occurred after an anaphylactic reaction to a wasp sting. This patient had a serum tryptase level of 25 ng per milliliter. Another subject (Patient 4) presented with chest pain, dyspnea, and mild obtundation and was admitted to the coronary care unit, where a myocardial infarction was ruled out and an exercise stress test was normal. Late in her hospital course, serum obtained on admission was found to have been positive for tryptase (38 ng per milliliter), indicating the probable anaphylactic nature of her precipitating event. Patient 5 had undetectable levels of tryptase before the procedure to desensitize her to horse antilymphocyte immunoglobulin. The patient with systemic symptoms after aspi-

rin ingestion also had an elevated level of tryptase (14 ng per milliliter).

The 17 patients with systemic mastocytosis had no acute cardiopulmonary or cutaneous manifestations when samples were obtained. Tryptase levels averaged ( $\pm$ SE)  $15.9 \pm 6.2$  ng per milliliter, and histamine levels averaged  $2.6 \pm 0.5$  ng per milliliter. In 6 of 17 patients, tryptase levels were undetectable. In the 11 patients with detectable levels of tryptase, the mean weight ratio of tryptase to histamine was  $8.8 \pm 2.3$ . The correlation of tryptase and histamine concentrations from all samples is shown in Figure 2. Regression analysis revealed a linear relation ( $P < 0.01$ ) with a slope of 6.6 and an intercept near the origin.

The time course of the disappearance of tryptase after an anaphylactic episode was analyzed in the four cases for which follow-up samples were obtained. Samples obtained 24 hours after a reaction to penicillin, wasp venom, or exercise contained levels of tryptase under 5 ng per milliliter. In Patient 4, who had acute systemic anaphylaxis after eating honeydew melon, the tryptase level had decreased from 39 to 18 ng per milliliter after a six-hour interval.

### DISCUSSION

Elevated tryptase concentrations were found in serum obtained after anaphylactic reactions in all of six

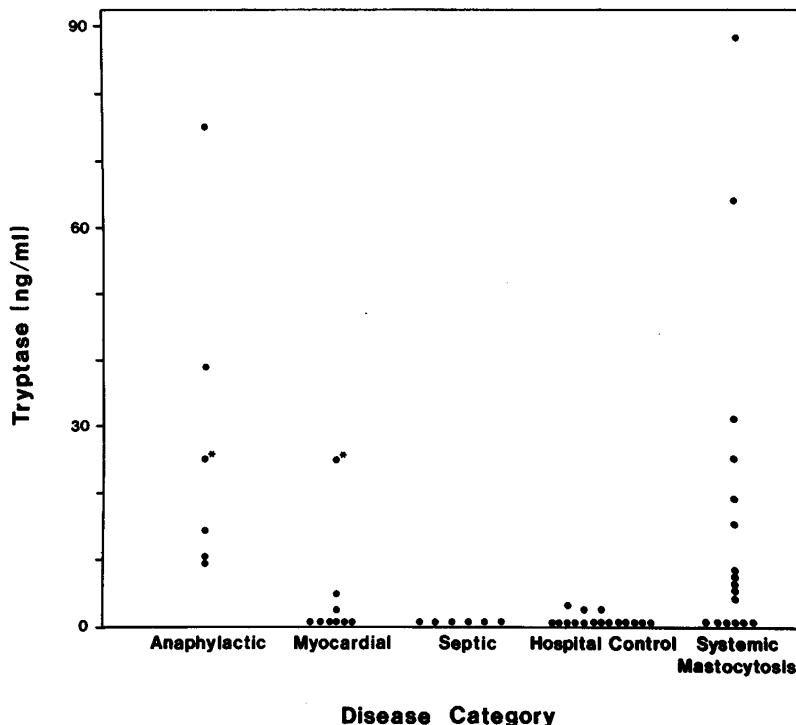


Figure 1. Levels of Tryptase in Patients with Anaphylaxis (N = 6), Myocardial Disease (N = 9, 4 with Shock), Sepsis (N = 6, 3 with Shock), and Systemic Mastocytosis (N = 17) and in 16 Controls.

Serum was used to measure tryptase in all subjects other than those with systemic mastocytosis, in whom plasma was used. Each value represents the average of two or more determinations. The asterisks indicate two points for the same patient, who had both a myocardial infarction and anaphylaxis.

subjects, indicating mast-cell involvement in this clinical condition. Plasma tryptase levels were also elevated in most patients (11 of 17) with systemic mastocytosis (even though they were not acutely symptomatic) and were linearly related ( $P < 0.01$ ) to the elevated levels of histamine. This linear relation was probably due to the mastocytosis cell's being the chief source of each mediator in these patients and to the achievement, in part, of a steady-state equilibrium between the secretion and removal of each mediator. Basophil concentrations are normal in subjects with systemic mastocytosis. Plasma<sup>4,6,7</sup> and urinary<sup>6,30</sup> histamine levels are usually elevated in such patients. The elevations in the levels of tryptase together with those of histamine indicate ongoing release from mast cells in patients with mastocytosis.

The specificity of tryptase as a marker for human mast cells has been shown in previous studies by immunohistochemical techniques.<sup>15-19</sup> Because tryptase is a preformed mediator, stored along with histamine and proteoglycan in the secretory granules of mast cells, the degranulation of mast cells, whether by immunologic or nonimmunologic activation, is accompanied by the release of this enzyme. For example, the anaphylactoid symptom complex that occurs after aspirin ingestion in sensitive persons<sup>31</sup> has a non-immunologic mechanism, and IgE antibodies directed

against aspirin have not been detected in sensitive patients.<sup>32</sup> Mast-cell involvement in aspirin-induced reactions has been suggested previously by the concomitant finding of increased levels of prostaglandin D<sub>2</sub> and its metabolites.<sup>33</sup> Prostaglandin D<sub>2</sub> is preferentially,<sup>34-36</sup> though not exclusively,<sup>12,13</sup> generated by activated mast cells. A metabolite of prostaglandin D<sub>2</sub>, presumably derived from mast cells, is elevated in the urine of patients with systemic mastocytosis,<sup>10</sup> and a metabolite of prostaglandin D<sub>2</sub> from alveolar macrophages or mast cells or both is elevated in bronchoalveolar lavage fluid after allergen challenge.<sup>11</sup> An abnormal platelet response to aspirin and indomethacin in aspirin-sensitive patients with asthma has also been observed,<sup>37</sup> suggesting that platelets may be an alternative source of prostaglandin D<sub>2</sub> in aspirin-sensitive patients. However, the elevated level of tryptase in one subject (Patient 6) with aspirin-induced bronchospasm and urticaria was consistent with its being a mast-cell-mediated event.

The mean ( $\pm$ SD) weight ratio of plasma tryptase to histamine in the

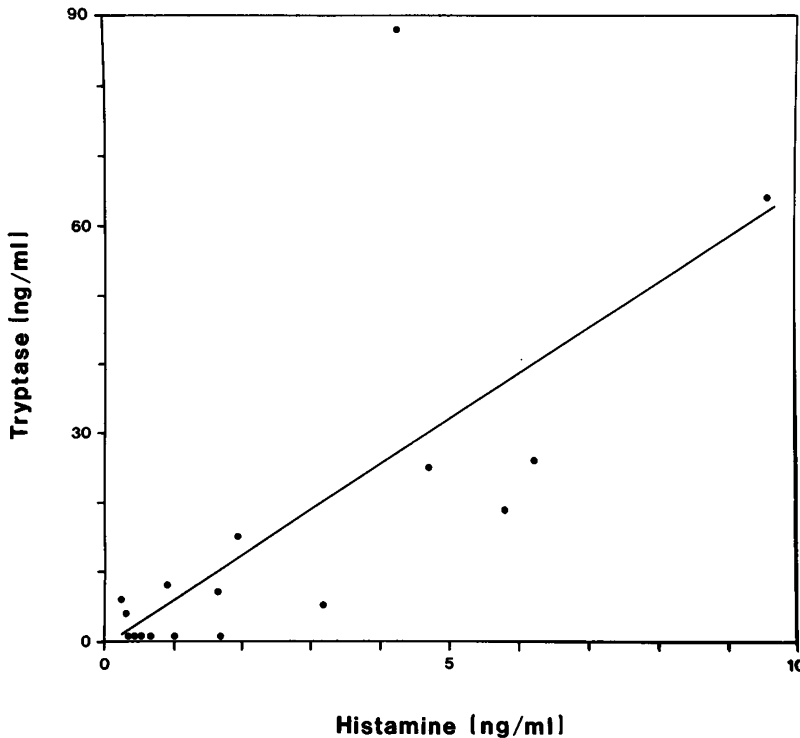


Figure 2. Correlation of Plasma Tryptase Concentrations with Those of Histamine in 17 Patients with Systemic Mastocytosis.

Each value reflects the average of two or more determinations made in a single patient.

11 patients with mastocytosis in whom tryptase was detected was  $8.8 \pm 2.3$ . This ratio is similar to that of  $8 \pm 4$  found in extracts of dispersed human-lung mast cells (predominantly T mast cells)<sup>20,24</sup> and that of  $18 \pm 11$  found in extracts of dispersed skin mast cells (predominantly TC mast cells).<sup>16</sup> It is unlikely that the serum or plasma weight ratio will distinguish the involvement of one type of mast cell from that of another. However, this ratio clearly indicates mast-cell involvement, because the ratio in basophils is only about 0.03.<sup>19</sup> Although weight ratios of tryptase to histamine in anaphylaxis have not been determined directly, peak plasma histamine levels of 20 to 140 ng per milliliter have been reported in exercise-dependent<sup>3</sup> and venom-induced<sup>2</sup> anaphylaxis and can be compared with the plasma tryptase levels of 9 to 75 ng per milliliter (probably not peak levels) in the current study. Variations in the relative concentrations of tryptase and histamine in plasma during allergic reactions could reflect variations among patients in the ratio of tryptase to histamine in mast cells, the relative contribution of histamine and tryptase from each type of mast cell and from basophils, and the different rates of metabolism, excretion, and diffusion from tissue into the circulation of these two mediators.

One advantage of using tryptase instead of histamine or histamine metabolites as an indicator of anaphylaxis is the ability to detect elevations in the tryptase

level several hours after the precipitating event. Although plasma histamine levels rise after anaphylaxis in response to bee venom,<sup>2</sup> exercise,<sup>3</sup> anesthetic medications,<sup>8</sup> and radioccontrast dye<sup>30</sup> (with patients being monitored closely), histamine levels return to normal 20 to 60 minutes after immunologic<sup>2</sup> or nonimmunologic<sup>3</sup> stimulation. Levels of histamine and histamine metabolites in urine also increase after anaphylactic events<sup>8,29,38</sup> and may remain elevated over a longer period (depending to a great extent on when the patient last voided). In the current study, the five patients with anaphylaxis that began outside the hospital arrived at the emergency room between one and three hours after the precipitating event, and all had elevated levels of tryptase (10 to 75 ng per milliliter). In Patients 1 through 3, the tryptase level had returned to base line after 24 hours; in Patient 4 the level decreased by 53 percent after six hours, suggesting a half-time of several hours for disappearance from the circulation. Alternatively, the prolonged presence of allergen

with persistent stimulation of mast cells should be considered. The apparent prolonged presence of tryptase relative to histamine in plasma or serum is probably due both to a more rapid rate of removal of histamine and to a slower rate of diffusion of tryptase from its major tissue sites of release into the circulation. The latter mechanism has been shown to occur after cutaneous allergen challenge, in which the time of appearance in skin chamber fluid of peak levels of tryptase (30 to 60 minutes) was delayed relative to that of histamine ( $\leq 30$  minutes).<sup>22</sup> This delay presumably reflected the slower rate of diffusion through tissue of the macromolecular tryptase-proteoglycan complex than of histamine, since both are released together from the secretory granules of mast cells. One disadvantage of measuring tryptase by the current method instead of measuring histamine is that the level of tryptase in control serum is normally below the limit of detection (2.5 ng per milliliter) and small elevations in tryptase may therefore not be detected.

A final advantage of tryptase over histamine as an indicator of anaphylaxis is that serum can be used to measure tryptase but not histamine. Histamine is released from basophils during blood clotting, which may falsely elevate the measured levels of histamine in serum; the tryptase level was always less than 5 ng per milliliter in serum samples from control patients and was always less than the level in serum samples

from those with systemic anaphylaxis, presumably because of the negligible amounts of tryptase present in basophils. This factor proved to be useful in Patient 4, who was admitted for presumed myocardial disease; only several days later was the serum sample that had been obtained on admission retrieved and a correct diagnosis of anaphylaxis established, based on an elevated tryptase level. In addition, ingestion of histamine-containing foods reportedly may be reflected in an elevation of histamine and its metabolites in the urine.<sup>39</sup>

In summary, tryptase is a distinguishing marker of human mast cells and, when it is detected in serum or plasma, indicates that mast cells are an active participant. Neither sepsis nor myocardial infarction alone (with or without concomitant hypotension) results in consistently detectable levels of tryptase in serum, though an associated anaphylactic event is reflected by an elevation in serum or plasma tryptase. Detectable levels of tryptase may persist for at least several hours, depending on the magnitude of the initial elevation and the duration of antigenic stimulation. The level of tryptase in the circulation appears promising as a diagnostic correlate in the evaluation of mast-cell-mediated disease.

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