

Markers of Mast Cell Degranulation

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Mast cells are the primary effector cells of immediate hypersensitivity reactions in humans. Upon mast cell activation both preformed and newly synthesized mediators are secreted. Histamine can be measured by fluorometric assays, radioenzymatic assays, and immunoassays. These methods have been applied to plasma and urine to detect histamine that had been released *in vivo* and to release histamine *in vitro* from basophils and mast cells. Another mast cell constituent is tryptase, which is a more selective marker of mast cells, because negligible amounts are found in basophils. β -Tryptase is stored in secretory granules and is actively released when mast cells degranulate. α -Protryptase remains in the proenzyme form and is constitutively released from mast cells, and consequently its level in serum reflects total numbers of mast cells. α -Protryptase levels are elevated in serum at baseline in subjects with systemic mastocytosis, whereas β -tryptase is elevated in serum from subjects with systemic anaphylaxis. These markers serve as precise clinical indicators of the involvement of mast cells in human disease. © 1997 Academic Press

blast growth factor (bFGF) also appears to be a preformed mediator of human mast cells (2). When mast cells are activated, newly formed mediators include the lipids, leukotriene C₄ (LTC₄), prostaglandin D₂ (PGD₂), and cytokines such as tumor necrosis factor (TNF)- α and interleukins (ILs)-4, 5, 6, and 13. Mast cells have been characterized by their proteinase contents into two types (3). MC_{TC} cells contain tryptase, chymase, mast cell carboxypeptidase, and cathepsin G, while MC_T cells contain tryptase and not the other proteases. The MC_{TC} cell is the predominant type of mast cell in skin and intestinal submucosa, whereas the MC_T cell is the predominant mast cell type in small bowel mucosa and alveolar walls. By measuring levels of the preformed mediators of mast cells in blood or other body fluids with specific, sensitive, and practical assays, mast cell activation and/or mast cell numbers in various clinical settings can be assessed. This article will focus primarily on two mediators, histamine and tryptase, as clinical markers of mast cells.

The mast cell is the primary instigator of immediate hypersensitivity reactions in humans (1). Mast cells are widely distributed throughout the body in connective tissues, in mucosal tissues, and juxtaposed to blood vessels. Activated mast cells release both preformed and newly synthesized mediators that shape the host response. The preformed mediators include the biogenic amine histamine; proteoglycans heparin and chondroitin sulfate E; and several proteases, including tryptase, chymase, mast cell carboxypeptidase, and cathepsin G. Basic fibro-

MAST CELL MEDIATORS

Histamine

Mast cells and basophils have several features in common. They are the two principal reservoirs of the biogenic amine, histamine (110 Da), which is formed within each cell type from histidine by histidine decarboxylase. Both cell types demonstrate metachromasia when stained with basic dyes, such as toluidine blue, giving a vivid purple color in tissue sections or smears of peripheral blood. An abundant

amount of functional surface FcεRI, the high-affinity receptor for immunoglobulin E (IgE), is expressed by each. When allergen bridges IgE bound to FcεRI on the cell surface, the contents of the secretory granules are released into the extracellular space, a process called degranulation. Newly generated mediators also are secreted. Although mast cells reside in tissues and basophils typically in blood, the histamine in plasma can be from either cell type. Plasma histamine levels reflect activation or numbers of these cells *in vivo*, irrespective of the atopic status of the individual. Basophils isolated from whole blood retain the IgE molecules bound to them *in vivo*. Histamine released from such cells in response to an *in vitro* allergen challenge reflect the presence of antigen-specific IgE. Consequently, basophil histamine release assays (BHR) correspond to other tests for antigen-specific IgE, such as skin tests. Because histamine measurements can be used to assess activation of mast cells and basophils *in vivo* as well as IgE sensitivities in BHR assays *in vitro*, numerous methods to measure this compound both in plasma and from isolated basophils have been developed through the years.

Methods for Measuring Plasma/Serum Histamine

Using histamine levels in blood as a clinical marker of mast cell and/or basophil involvement poses certain difficulties. Histamine has a plasma half-life of about 2 min—so timing is important (4). Hemolysis, venous stasis, coagulation, cell injury secondary to rapid blood drawing or centrifugation, or improper storage of blood samples can lead to misleading, artifactual histamine elevations. Additionally, ingestion of a histamine-rich food can affect plasma histamine levels (5). Nevertheless, methods have been developed to measure histamine. These include bioassays, fluorometric assays, radioimmunoassays (RIA), and radioenzymatic assays.

The earliest bioassay (1946) for histamine used strips of ileum from sensitized guinea pig (6). Following antigen exposure, the contractile response was measured in proportion to the histamine concentration. This method, though sensitive, was laborious, and results were difficult to standardize among different laboratories.

In 1959 a spectrophotometric method that measured the monodinitrophenyl derivative of histamine formed in a reaction with 2,4-dinitrofluorobenzene (DNFB)(7) was developed. This method was sensitive to 5–10 ng/ml. However, interfering sub-

stances had to be removed before the reaction was performed.

In 1961, Noah and Brand developed a more sensitive method (1 ng/ml) in which histamine was coupled to *o*-phthalaldehyde (OPT) at an alkaline pH to form a fluorescent product (8). However, an extraction procedure was still necessary to remove histidine and other possible interfering compounds.

The fluorometric method was automated in 1974 by Siraganian, allowing greater throughput, though certain contaminating compounds still interfered (9). The sensitivity of the automated fluorometric assay was 250 pg/ml and allowed 20–30 samples/h to be assayed with excellent accuracy and reproducibility. It offered major advantages for assaying histamine levels after stimulation of basophils or mast cells *in vitro*. However, plasma histamine levels in normal subjects range from about 100 to 500 pg/ml. Consequently, plasma histamine levels are not in the detectable range in a portion of normal subjects.

Radioenzymatic assays were developed in parallel with the fluorometric assays. One of the first of such assays was described by Snyder *et al.* in 1966 (10). The single-isotope method involved the enzymatic transfer of a [¹⁴C]methyl group from S[¹⁴C]adenosylmethionine-(¹⁴C]SAM) to histamine, forming [¹⁴C]-methylhistamine. The enzyme responsible for this transfer, histamine methyltransferase, was not commercially available and had to be purified from male guinea pig brains or rat kidneys. The sensitivity of this early assay was <1 ng/ml. Later a trace amount of [³H]histamine was added to the assay (double-isotope method) to correct for variabilities in the extraction of [¹⁴C]methylhistamine (11). In this case, the final product was [¹⁴C]methyl [³H]histamine. A standard curve was determined by incubating known amounts of histamine with the enzyme. Gleich and Hull reported that there could be large differences in histamine measurements among different laboratories using both the radioenzymatic and the fluorometric methods (12). Several groups made adjustments in the radioenzymatic assay to improve its ability to measure plasma histamine and to increase sensitivity and reproducibility (13–18). These changes improved sensitivity to 30 pg/ml, making it possible to detect plasma histamine levels in nearly everyone. Normal plasma levels of histamine averaged about 300 pg/ml. Although the radioenzymatic assays are the most sensitive, they are complex and time-consuming, particularly because individual laboratories had to produce the transfer enzyme.

Immunoassays now available for histamine measurements have eliminated many of the problems associated with fluorometric and radioenzymatic assays (19–22). The immunoassays are commercially available in both enzymatic and radioactive formats. Because of low complexity, they can easily be performed in nonresearch settings. With sensitivities of about 100 pg/ml, plasma histamine levels for most individuals are in the detectable range of the assays.

In the late 1980s, an assay was developed based on the binding of histamine to borosilicate glass (23). In this method histamine released *in vitro* from antigen- or anti-IgE-stimulated basophils was incubated with and adsorbed to glass microfibers. After the microfibers were washed, histamine was desorbed with HClO₄, coupled to OPT, and measured in a spectrofluorometer.

Histamine itself is small and not immunogenic. Guesdon linked histamine to 1,4-benzoquinone and after immunizing mice, a monoclonal antibody (mAb) to histamine was obtained and used to develop an ELISA (19). This assay exhibited a high degree of specificity, because the mAb did not recognize histidine, methylhistamine, or various drugs known to interfere with fluorometric assays for histamine. It had a sensitivity of 45 pg/ml and correlated well with results obtained using a fluorometric method (20). In 1988 another group developed a mAb to histamine that had been acylated (21). In this case, acylated histamine in the sample competed with a standard amount of ¹²⁵I-acylated histamine for binding to the mAb. This immunoassay had a sensitivity of 10 pg/ml of histamine. McBride *et al.* evaluated several plasma and urine samples and found that this new RIA correlated well with a radioenzymatic assay using thin-layer chromatography (24).

Urinary Histamine Levels

Histamine can be measured in urine as well as in plasma. Studies of urinary metabolites of subcutaneously administered [¹⁴C]histamine in human demonstrated that 50% of histamine is excreted as 1-methylimidazole-4-acetic acid (1,4-MeImAA), 30% as imidazoleacetic acid, 5% as 1,4-methylhistamine, and 2% as nonmetabolized histamine (25). Because 1,4-MeImAA is the main metabolite of histamine in the urine, its concentration normalized to that of creatinine reflects endogenous release of histamine. Levels of imidazolic acids can be measured by ELISA, mass spectrometry, radioimmunoassay, or

gas chromatography (25–28). *N*-Methylhistamine levels in urine measured by radioimmunoassay peak 1 h after IV infusion of histamine and decrease to resting levels after 2 h (28). Genitourinary bacteria may be capable, however, of converting histidine to histamine and falsely elevating urinary levels of histamine and its metabolites (29). Consequently, the normal range of urinary histamine or histamine metabolite levels is quite large.

Basophil Histamine Release

In 1940–1941 Katz and Cohen described histamine release *in vitro* from leukocytes isolated from the peripheral blood of sensitive individuals and challenged with allergen (30, 31). In 1964 Lichtenstein and Osler suggested that *in vitro* leukocyte (basophil) histamine release to an allergen challenge correlated to *in vivo* allergic sensitization (32). The basophil histamine release (BHR) assay demonstrated the presence of antigen-specific IgE bound to functional FcεRI on the surface of basophils and exhibited a sensitivity and specificity comparable to allergen skin tests and an allergen bronchial challenge. Although initial studies utilized unfractionated leukocytes, later studies used enriched preparations of basophils. The complexities of isolating, maintaining, and stimulating basophils and of measuring histamine have limited the routine use of basophil histamine release assays in diagnostic labs. Skin tests or direct measurements of antigen-specific IgE in serum remain the preferred methods for evaluating atopic sensitivities.

Clinical Aspects

Histamine levels in human mast cells (both MC_{TC} and MC_T types) and basophils range from 1 to 3 pg/cell (33). In resting cells, histamine is stored in secretory granules. At the acidic pH of granules in such cells (34), histamine is positively charged and bound to carboxyl groups on proteins or proteoglycans. When mast cells are activated, histamine dissociates from the proteoglycan matrix in exchange for extracellular sodium at neutral pH (35). Once released into the extracellular space, histamine freely diffuses into the surrounding tissue and into the circulation where it is rapidly metabolized. Histamine metabolic pathways are depicted in Fig. 1. Histamine has been measured in several body fluids including synovial fluid, tears, nasal secretions, bronchial washings, urine, and skin chambers, where it reflects local basophil or mast cell involvement. Ele-

vated histamine levels in plasma occur following severe allergic reactions, such as systemic anaphylaxis (34), and at baseline in many patients with systemic mastocytosis (36–38). During anaphylaxis to insect sting challenge, histamine levels peak approximately 5 to 15 min after the sting and return to baseline by 10 to 30 min later (34).

Basophil histamine release assays from antigen-challenged, sensitized, peripheral blood basophils have been touted as an alternative to skin testing to measure allergen sensitization, but they have not replaced the sensitivity, specificity, cost effectiveness, and practicality of skin tests. Thus, histamine measurements are a useful research tool, but in general are not a practical diagnostic tool.

Tryptase

There are several proteases present in mast cell secretory granules, including β -tryptase, chymase,

mast cell carboxypeptidase, and cathepsin G. β -Tryptase is the principal enzyme in all mast cells, accounting for up to 20% of the protein content (39). Mast cells in lung and skin contain on average 11 and 35 pg β -tryptase/cell, respectively (40). Basophils contain only 0.05 pg/cell (33). Currently, only mast cells and basophils have been shown to express tryptase mRNA, with basophils having 10^5 -fold less than mast cells (41). β -Tryptase is a serine endoprotease that has trypsin-like activity (42). It exists in its active form as a tetramer in ionic association with heparin. Each subunit of the tetramer has one active site. Traditional serine protease inhibitors do not inhibit tryptase activity. Under physiologic conditions, β -tryptase is active only as a tetramer, and once dissociated from heparin, it spontaneously converts to inactive monomers, a process that appears to be biologically irreversible

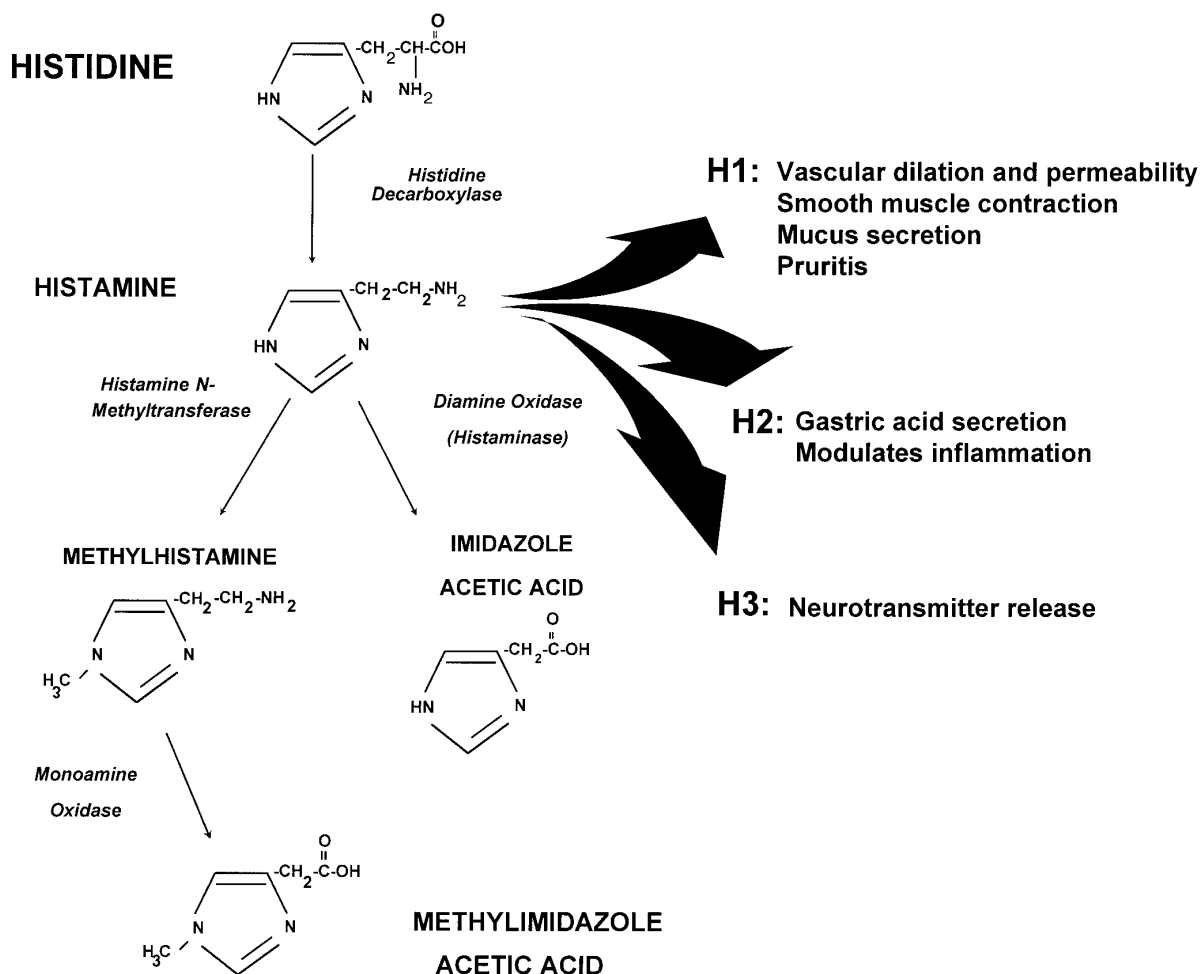


FIG. 1. Synthesis and metabolism of histamine.

(43). Although β -tryptase exhibits a neutral pH optimum against small synthetic peptide substrates, acidic pH optima were found for its processing of protein substrates such as β -protryptase (44), low-molecular-weight kininogen (45), and fibrinogen (46). An acidic pH optimum for biologic protein substrates may favor activity at local sites of inflammation, wound healing, or neovascularization.

There are at least two tryptase genes, α -tryptase and β -tryptase, each of which resides in the normal haploid genome on chromosome 16 (47). Messenger RNAs for α -tryptase (48) and β -tryptase (47, 49) show 92% homology for the encoded proteins. β -Preprotryptase (30-amino-acid leader peptide, 245-amino-acid catalytic portion) is processed from an inactive monomeric form to a fully active tetramer by a two-step pathway (Fig. 2) in the presence of heparin at an acidic pH (44). First the prepro portion is converted to the proform by removing the 18-amino-acid signal peptide. The monomeric β -protryptase then undergoes heparin-dependent auto-

catalytic cleavage (at an acidic pH) between Arg⁻³/Val⁻² amino acids, thus creating the β -pro'tryptase form. α -Protryptase has a substitution of Gln⁻³ for Arg⁻³ that prevents autoprocessing, and α -protryptase therefore remains in the proenzyme state where it is constitutively secreted. Consequently, α -protryptase is found in blood in normal subjects. β -Pro'tryptase has the pro' dipeptide removed by DPPI (dipeptidyl-peptidase I) to yield an inactive monomer of mature tryptase. DPPI is a cysteine-type processing protease that functions at acidic pH values and is found in all bone marrow-derived granulocytes. In the presence of heparin, the monomers come together on a heparin backbone to form an active tetramer. These events probably take place in the Golgi and post-Golgi secretory progranules in the mast cell. The mature packaged tryptase is then stored until mast cell activation causes the granular contents to be released.

The precise pathophysiologic actions of tryptase have not been completely discerned. However, the protease may play an important role in tissue turnover during inflammation. Biologically active tryptase (β -tryptase) has been shown to cleave and inactivate fibrinogen (46, 50). It generates the anaphylatoxin C3a from C3 (51) and destroys various neuropeptides, including VIP and CGRP (52). It activates prostromelysin (metalloproteinase III), a potential activator of procollagenase (53). Tryptase also enhances the contractile effects of histamine on airway smooth muscle, an activity of potential relevance to airway hyperreactivity in asthma (54). Tryptase stimulates proliferation, chemotaxis, and collagen production by fibroblasts, activities that are potentially fibrogenic (55–57). The enzyme stimulates epithelial cell proliferation as well as IL-8 production, suggesting a role for tryptase in epithelial remodeling and recruitment of inflammatory cells following mast cell activation in asthma (58). Stimulation of dermal microvascular endothelial cells to proliferate and form tubes suggests a role for tryptase in neovascularization (59).

Several assays have been developed to measure tryptase levels in biological fluids. In fluids other than serum or plasma, normal values must be determined in the context of each experimental design. Several mouse mAbs and goat polyclonal Abs against tryptase have been produced. The first immunoassay developed using these antibodies was a sandwich ELISA (22). The mAb G5, which binds β -tryptase or β -protryptase at least 10-fold better than α -protryptase (60), was used to capture tryptase

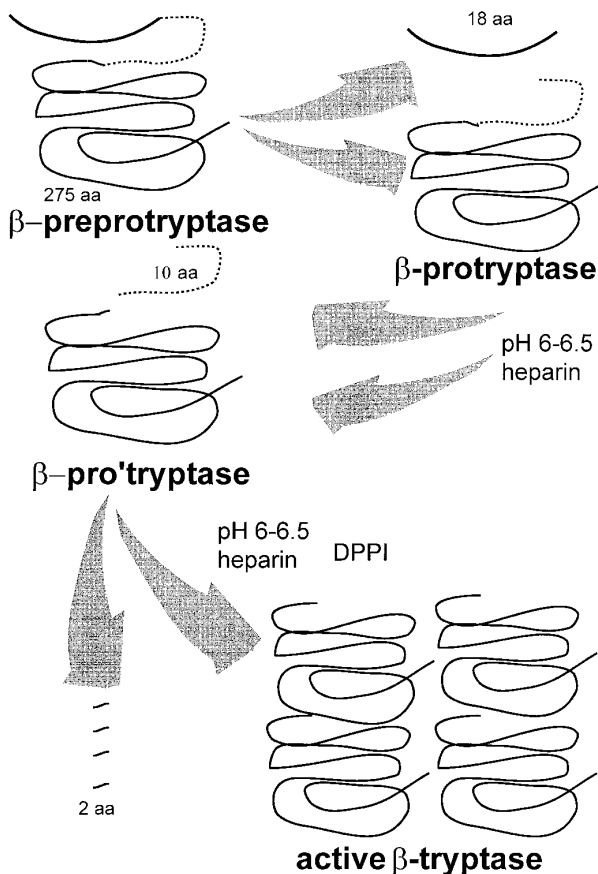


FIG. 2. Processing of human tryptase.

tase. The captured protease was then detected with goat IgG anti-tryptase and an alkaline phosphatase-conjugated swine anti-goat IgG. G5 recognizes a linear determinant available on inactive or denatured β -tryptase. The sensitivity of this assay was 2.5 ng tryptase/ml of serum or plasma. More sensitive assays (1 ng/ml) were developed that used G5 for capture and another noncompetitive mAb, G4, for detection. G4 was either biotinylated, in which case alkaline phosphatase–streptavidin was used to develop the assay (61), or radiolabeled with ^{125}I (62). G4 recognizes a conformational epitope that is present on active tetrameric β -tryptase as well as inactive monomeric β -tryptase and α -protryptase.

Using the G5/G4 assay, β -tryptase is essentially undetectable in normal serum and plasma (<1 ng/ml). β -Tryptase values that increase to ≥ 1 ng/ml during a clinical event suggest that mast cell degranulation has occurred. β -Tryptase measured by the G5 immunoassay has been commercially available as a radioimmunoassay from Pharmacia–Upjohn.

Another tryptase immunoassay that detected β -tryptase and α -protryptase about equally was developed (63). The mAb B12 was used for capture, and biotin–G4 with alkaline phosphatase-conjugated streptavidin was used for detection. This assay measures total tryptase. Levels in normal serum and plasma are equal and range from 1 to 10 ng/ml. Using both the G5 immunoassay (predominantly β -tryptase) and the B12 immunoassay (α -protryptase and β -tryptase), the amount of α -protryptase can be estimated from the difference of the two assays (60). Since α -protryptase is constitutively released from mast cells, levels of this form of tryptase reflect total mast cell numbers and appear to be useful for the diagnosis of patients with systemic mastocytosis.

TABLE 1

Selective Properties of α -Protryptase and β -Tryptase

Property	α -Protryptase	β -Tryptase
Enzymatic activity	–	+
Secretory granule/degranulation	–	+
Constitutive secretion	+	–
B12 mAb binding	++	++
G5 mAb binding	–/+	++
Systemic mastocytosis	+++	–
Systemic anaphylaxis	–	+++

Note. Symbols + or – represent the differential levels of the properties indicated.

Various features of the two assays are summarized in Table 1.

Other laboratories have developed assays to measure tryptase release from mast cells. One such assay from Lavens *et al.* developed a colorimetric assay that used the trypsin-like activity of tryptase to measure nitroaniline liberated from benzoyl-DL-arginine-*p*-nitroaniline (64). A strong correlation was demonstrated between tryptic activities and the levels of tryptase measured by radioimmunoassay. Because this assay measures enzymatically active tryptase, only β -tryptase is measured. Another assay used mAbs to tryptase to develop a competitive ELISA to measure levels in body fluids (65). This assay appears to measure total tryptase.

Tryptase Levels as a Clinical Tool

Anaphylaxis. β -Tryptase levels have become a useful diagnostic tool indicating mast cell activation. When mast cells degranulate, tryptase is released in parallel to histamine (66), but diffuses into the blood more slowly than histamine (67). This is because tryptase resides in a macromolecular protease:proteoglycan complex. During anaphylaxis from a bee sting, tryptase levels are maximal about 1 h after the sting and then decline with a half-life of about 2 h. This is in contrast to histamine, which has a half-life of about 2 min. This difference allows tryptase levels to be measured up to several hours after mast cell degranulation. Normal β -tryptase levels are <1 ng/ml using the G5 capture assay and are typically ≥ 5 ng/ml during hypotensive systemic anaphylaxis induced by an insect sting. Serum levels of β -tryptase correlate with the severity of hypotension that occurs during insect sting-induced anaphylaxis (68).

β -Tryptase levels have been used to determine whether systemic anaphylaxis occurred near the time of death by evaluating postmortem serum. Levels >10 ng/ml appear to reflect substantial antemortem mast cell activation, because levels were not elevated in 57 sequential sera collected postmortem from six control subjects (69). This suggests that β -tryptase levels could be used as a practical indicator of fatal mast cell-dependent anaphylaxis. Contrasting the diagnostic specificity of postmortem tryptase levels is a study that found levels of β -tryptase that were >10 ng/ml in 5 of 49 cases thought to be nonanaphylactic deaths (70). One was a salicylate overdose (23 ng tryptase/ml). Because mast cell activation occurs with anaphylactic and airway hyper-

sensitivity reactions to cyclo-oxygenase inhibitors, this subject could have been aspirin-sensitive. Another had a diagnosis of atherosclerotic coronary vascular disease (33 ng tryptase/ml), as did 10 other subjects with levels <5 ng/ml. Details regarding drugs received near the time of death, particularly those that might activate mast cells such as morphine, were not available. Three subjects died of multiple trauma (20, 24, and 106 ng tryptase/ml). Thus, a careful consideration of the events near the time of death may be needed to fully interpret post-mortem levels of tryptase.

Interestingly, β -tryptase (by G5 assay) levels are not elevated in some cases of severe food-induced anaphylaxis, suggesting a non-mast-cell-dependent mechanism in these instances (69, 71). Two alternative considerations are complement-mediated and basophil-mediated anaphylaxis. β -Tryptase levels are elevated during aspirin and nonsteroidal anti-inflammatory drug (NSAID)-induced anaphylaxis (67, 72). β -Tryptase levels have also been used to investigate hypotensive episodes occurring during induction of general anesthesia (73–75).

An issue of practical concern is sensitivity of total tryptase (B12 capture assay) compared to β -tryptase (G5 capture assay) for mast cell-dependent systemic anaphylaxis. It appears that a single determination of total tryptase, clinically, will be less sensitive than a single determination of β -tryptase (63). However, if baseline and acute blood samples are compared, a twofold or greater increase in total tryptase during the acute event will provide at least as high a sensitivity as the β -tryptase-specific assay. Baseline serum or plasma should be obtained from blood collected either prior to the acute clinical event or at least 24 h after its resolution.

Sudden infant death syndrome. Anaphylaxis has been postulated as a cause of sudden infant death syndrome (SIDS) in a subset of infants (76–78). The availability of tryptase as a marker of mast cell activation permitted a direct assessment of postmortem sera for evidence of premortem anaphylaxis. In one study, 40% of 50 infants who died from SIDS were found to have elevated β -tryptase levels (>10 ng/ml), suggesting possible mast cell involvement in a subset of crib death victims (79). None of the control infants who died from other causes were found to have elevated tryptase levels. However, no statistically significant association of antigen-specific IgE and β -tryptase was present in either group. The postmortem interval did not affect tryptase levels.

Also, there was no increase in the numbers of mast cells in the tissues of the SIDS group. Another study also found elevated tryptase levels in SIDS compared to explained death controls (80). The mechanism of mast cell activation remains unknown.

Systemic mastocytosis. Mastocytosis is associated with increased mast cell numbers in skin, liver, spleen, lymph nodes, and bone marrow (81). There are four main types of mastocytosis: (i) skin involvement only, called urticaria pigmentosa, (ii) indolent systemic mastocytosis, (iii) systemic mastocytosis associated with a hematologic disorder, and (iv) aggressive systemic mastocytosis (82). Serum α -protryptase levels were markedly elevated (>20 ng/ml) in patients with systemic disease, but not in those with local skin disease alone, presumably reflecting the total mast cell burden (60). In such cases, the serum level of β -tryptase (G5 capture assay) should be at least 10-fold lower than that of α -protryptase (total tryptase assay). Baseline levels of α -protryptase did not differ among groups 2 to 4, nor did they vary among group 2 subjects with varying degrees of clinical complications from the disorder. The serum level of α -protryptase serves as an excellent test for diagnosing systemic mastocytosis. It also can be used to assess the response to therapies designed to lower the mast cell burden. In three patients with systemic mastocytosis receiving interferon- α 2B for at least 12 months, no significant change in either α -protryptase levels or clinical course was found (83).

Other allergic diseases. Tryptase levels in biological fluids outside of blood can reflect local mast cell activation when appropriate control levels are obtained. Levels measured in bronchoalveolar lavage fluids collected during the early and late responses to allergen challenges show elevated tryptase levels early (12-min sample), but not late (48 h), suggesting mast cell degranulation only during the early phase of the response (84). BAL tryptase levels have also been measured in atopic patients with and without asthma as well as in patients who were nonatopic with and without asthma (85). In the atopic asthmatic group, baseline BAL tryptase levels were elevated compared to the other groups, suggesting chronic mast cell degranulation in atopic asthma. Tryptase levels were not elevated in BAL fluids during exercise-induced asthma, suggesting that no activation of mast cells above baseline occurs with exercise-induced asthma (86, 87).

β -Tryptase has also been measured in nasal lavage

fluid (88) and in nasal fluid collected directly on gauze (89). Following an allergen challenge, tryptase levels in lavage fluids correlated better than histamine levels to clinical symptoms of the atopic response (88). Tryptase levels in nasal fluid were elevated in subjects with seasonal allergic rhinitis in-season but not out-of-season and not in nonatopic individuals, suggesting mast cell degranulation is an ongoing process during seasonal allergic rhinitis much like in the airway of asthmatic individuals (89).

OTHER MEDIATORS

Mediators other than histamine and tryptase can be considered as clinical markers of mast cell activation. For example, leukotriene C₄ (LTC₄) and prostaglandin D₂ (PGD₂) are both secreted by activated mast cells. PGD₂ levels in bronchoalveolar lavage fluid are elevated in subjects with atopic asthma (90). Urinary levels of a PGD₂ metabolite, measured using gas chromatography and mass spectrometry, are elevated in patients with systemic mastocytosis (91). PGD₂ levels measured in the urine of patients undergoing aspirin desensitization increase with systemic reactions (92). Although basophils do not produce PGD₂, platelets, Langerhans cells and other antigen-presenting cells contain PGD synthase and are able to generate PGD₂ (93, 94). Thus, the cell source of PGD₂ measured in biologic fluids may be uncertain. Sensitive and specific assays also exist for sulfidopeptide leukotrienes; however, these are not uniquely produced by mast cells and would not distinguish involvement of mast cells from other inflammatory cells such as eosinophils and basophils. Proteoglycans inside the mast cell have also been suggested as possible indicators of mast cell involvement. While heparin is uniquely stored and released from mast cells, currently there is no practical assay of sufficient specificity and sensitivity. Cathepsin G, a preformed mediator of the MC_{TC} type of mast cell, is also present in human neutrophils. Mast cell carboxypeptidase and chymase, also present in the MC_{TC} type of mast cell, are potential markers for this mast cell subset, but thus far no assays have been developed of adequate sensitivity and specificity for use with biologic specimens.

CONCLUSIONS

Mast cells are important cells in the inflammatory response. *In vitro*, where mast cells are the predomi-

nant cell type, most of the mast cell preformed and newly synthesized mediators can be used to assess mast cell activation. In more complex *in vivo* environments, histamine and PGD₂ or PGD₂-metabolite levels are sometimes helpful in assessing mast cell involvement, but there are ambiguities as to cell source. β -Tryptase levels are the most specific measure of mast cell activation in biologic specimens, whereas α -tryptase levels in the blood best reflect the number of mast cells in the body.

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