



Biomarkers of anaphylaxis, beyond tryptase

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Purpose of review

The purpose of this study is to describe the current knowledge regarding mediators involved in anaphylactic reactions, with a special focus on key effector cells and mechanisms.

Recent findings

New insight into the potential relevance of pathways other than mast cell degranulation has been unravelled, such as the role of cytokines, platelet activation factor, lipid mediators and their metabolism or the activation of the contact system.

Summary

Gaining knowledge into these pathophysiologic mechanisms will allow researchers to pursue the identification of risk factors and new preventive and therapeutic strategies in anaphylaxis.

Keywords

anaphylaxis, contact system, mast cell, mediators, tryptase

INTRODUCTION

Anaphylaxis is a multisystem syndrome that is rapid in onset and potentially fatal [1,2]. It most often represents an immunologic response, resulting from sudden systemic degranulation of mast cells and basophils [3].

Diagnosis of anaphylaxis is based on suggestive clinical symptoms after exposure to a potential triggering agent or event [1], given that no biomarker allows an unequivocal diagnosis of anaphylaxis. Subsequently, anaphylaxis remains under-recognized, undertreated and poorly understood.

Analysing mediators in human anaphylaxis is difficult because of the opportunistic nature of anaphylaxis, and the ethical issues surrounding deliberate provocation of anaphylaxis. In addition, interpretation of their usefulness as markers in anaphylaxis is also hampered by the fact that key steps in the allergic reaction occur locally and may not be detectable systemically.

This review summarizes the most recent knowledge of how production of particular mediators, depending on the mechanism of the anaphylaxis, may influence severity and clinical symptoms. It also discusses the usefulness of measuring mast cell tryptase and other mast cell mediators during anaphylaxis.

MECHANISMS AND EFFECTOR CELLS INVOLVED IN ANAPHYLAXIS

Triggers, pathophysiologic mechanisms and key effector cells involved in the anaphylactic reaction

will influence which mediators are released. This is summarized in Table 1.

IgE-MEDIATED ANAPHYLAXIS

In humans, anaphylaxis is predominantly due to immediate hypersensitivity reactions, initiated by an allergen interacting with allergen-specific IgE (sIgE) bound to its high-affinity receptor (FcεRI) expressed on effector cells, mainly mast cell and basophils [4].

Allergen/sIgE complexes initiate intracellular signalling that result both in release of preformed and de-novo synthesis of mast cell mediators [5]. Mast cells are found in the gastrointestinal tract, skin, lungs and heart. Production of particular mediators differs among mast cells in different tissue locations [6]. Therefore, the severity and symptoms of anaphylaxis may be partially dependent on the route of allergen exposure (i.e. ingested or injected), the tissue mast cell population activated [7] and

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KEY POINTS

- Anaphylaxis involves the activation of multiple immune pathways.
- No biomarker has been described that allows an unequivocal diagnosis of anaphylaxis.
- Future research for risk, prevention and treatment strategies will have to address multiple mediators of different pathways.

also on the mechanism that induces mast cell degranulation. In fact, the secretory granules storage of tryptase or other mast cell protease establishes a physical separation between IgE-induced mast cell degranulation, which engages the secretory granule compartment of large granules (0.5–1 μ m) and non-IgE response in which cytokines induce mast cell exocytosis and involves small secretion vesicles, usually 80 nm or less [8[¶]].

In humans, Fc ϵ RI complexes are expressed on several additional cell types, including monocytes/macrophages, Langerhans cells, myeloid and plasmacytoid dendritic cells, eosinophils, neutrophils, platelets and bronchial smooth muscle cells [5]. Their role in propagating the systemic manifestations of anaphylaxis cannot be overlooked. Basophils have been shown to be dispensable for IgE-mediated anaphylaxis but play a crucial role in IgG-mediated anaphylaxis in murine models [9]. Nevertheless, mechanistic studies have yet not been able to fully explain why some individuals have high levels of sIgE devoid of clinical reactivity, while others with undetectable or very low levels of sIgE can experience life-threatening reactions [7].

NON-IGE-MEDIATED ANAPHYLAXIS

Although IgE-mediated anaphylaxis is the most frequent, further immunologic and nonimmunologic mechanisms of anaphylaxis have been described [7,10].

IMMUNOLOGIC

Other non-IgE-mediated immunologic mechanisms of anaphylaxis are listed as follows.

Complement generation (anaphylatoxins, C3a and C5a)

Some substances, such as peanuts, have been described to have the ability to rapidly activate complement, with production of large amounts of the anaphylatoxin C3a. The rapid production of C3a through an antibody-independent pathway stimulates macrophages, basophils and, to a lesser extent, mast cells, to secrete platelet-activating factor (PAF) and histamine, which contribute to the induction of anaphylaxis by increasing vascular permeability [11].

Contact and coagulation system activation

At the end of 2007, more than 150 patients died from anaphylactic hypotension associated with intravenous heparin treatment [12]. Comprehensive analyses identified an unnatural contaminant in heparin that was characterized as oversulfated chondroitin sulphate [13]. Oversulfated chondroitin sulphate contaminated heparin has a greatly increased potency for activating FXII and triggering prekallikrein-mediated bradykinin formation in human plasma [14]. Activation of FXII-driven

Table 1. Main mechanisms, effector cells and mediators involved in human anaphylaxis

Type of anaphylaxis	Mechanism	Main effector cell involved	Main mediator involved
IgE-mediated	IgE bound to high-affinity receptor Fc ϵ RI	Mast cell and basophil	Histamine, tryptase, chymase, carboxipeptidase, heparin, PAF
Non-IgE-mediated			
Immunological			
Complement	C3a and C5a (innate response)	Macrophage, basophil, mast cell	Histamine, PAF
Contact system/Coagulation system	Kallikrein-FXII system	Endothelial cells	Bradykinin
IgG	IgG binding by FcRIIb and Fc γ RIII	Mast cell, macrophage, basophil	PAF
Nonimmunologic (physical factors, ethanol, morphine, codeine and others)	Directly	Mast cells	Histamine, tryptase, chymase, heparin, PAF

FXII, factor XII; Ig, immunoglobulin; PAF, platelet-activating factor.

contact system cascades leads to increased vascular permeability with oedema and hypotension.

IgG

It has been difficult to confirm the contribution of allergen-specific IgG to human anaphylaxis; it has been proposed that IgG-mediated anaphylaxis in humans requires considerably more antigen than IgE-mediated anaphylaxis, such as in reactions to infused drugs such as contrast media and antivenoms. This most likely reflects the much higher affinity of IgE binding by FcεRI than IgG binding by FcγRIII [15]. Khodoun *et al.* [15] explored various blood markers discerning between IgE and IgG-mediated anaphylaxis in mice to assess them in humans. Their observations suggested that decreased blood neutrophil FcγRIII expression without increased interleukin (IL)-4Rα expression would indicate IgG-mediated human anaphylaxis [15]. In mice, macrophages have been reported to play a major role in IgG-mediated systemic anaphylaxis through the release of PAF [16].

Basophils are not required in murine IgE-mediated anaphylaxis but are crucial in IgG-mediated anaphylaxis, through their release of PAF and their ability to bind immune complexes via the low-affinity IgG receptor FcγRIII. Previous studies have shown that the degree of basophil responsiveness may predict degranulation and severity of an allergic reaction [9].

NONIMMUNOLOGIC MECHANISMS

Some drugs, such as codeine and morphine, ethanol and physical factors such as exercise can induce mast cell degranulation directly [17].

MAST CELL MEDIATORS

Mediators of anaphylaxis traditionally have been divided into three classes: preformed mediators stored in the cytoplasmic granules released by degranulation such as histamine, the proteases tryptase and chymase, carboxypeptidase A and proteoglycans (with heparin as the major component); newly generated proinflammatory lipid mediators (i.e. prostaglandins, leukotrienes and PAF); and newly synthesized growth factors, cytokines and chemokines.

Subsets of mast cells have been described depending on the presence or absence of different serine proteases in their granules. MC_T (tryptase+/chymase-) tend to be located in the mucosa of organs; MC_{TC} (tryptase+/chymase+) are abundant in the skin perivascular tissue, heart, conjunctiva

and bowel submucosa and MC_C (tryptase-/chymase+) are very minor and unknown [18–20]. There are different modes of mast cell degranulation: piecemeal or anaphylactic [21]. Subsequently, the type of mast cell involved and the type of secretion mechanism will determine mediators released and their kinetics.

The two most abundant and well characterized performed granule mediators released by mast cells are tryptase and histamine. Currently, both are available for routine assessment, although not in a point-of-care fashion but for later assessment of anaphylactic reactions.

Table 2 summarizes the main mediators, indicating best sampling time, availability of commercial assays and correlation with anaphylaxis severity.

TRYPTASE

Mast cell tryptase belongs to the family of mast cell serine proteases and is present in large amounts in the secretory granules of human mast cells [22]. Tryptase is highly specific of mast cell and its detection provides information about mast cell distribution, number, activation status or releasability. Tryptase is also produced in basophils but in much lower amounts [23,24]. In healthy individuals, serum baseline tryptase levels are very stable over time [22]. Serum baseline tryptase levels provide information about potential underlying mast cells. Therefore, it is of paramount importance to perform sequential peak and baseline serum tryptase measurements.

Serum tryptase determination is highly valuable if correctly performed at adequate timing. Nowadays, it is the best routine biomarker available to assess mast cell activation. Levels are increased from 15 min to 3 h after anaphylaxis onset [19] or even longer [25], with the peak between 1 and 2 h [25]. Serial measurements of total serum tryptase have been shown to increase diagnostic sensitivity and specificity [26]. In addition, the difference or the ratio of tryptase during anaphylaxis and at baseline may aid in the diagnosis [27].

Measuring serum tryptase does not need special requirements regarding sample handling, the method is stable, robust and easy to process, and the time-frame for sampling is considerable. In some studies [25,28], serum tryptase concentration has been shown to correlate well with the severity of the anaphylaxis.

Nevertheless, tryptase is not an optimal biomarker because even when sampling in a correct time-point, it has been demonstrated that in a percentage of patients (36–40%), concentrations

Table 2. Main mast cell mediators in anaphylaxis

Mediator	Interval of recommended time sampling (peak time)	Commercial method availability	Correlation with severe anaphylaxis
Preformed mediators			
Histamine (plasma)	0–30 min (5–10 min)	Yes	Yes
Histamine (24-h urine)	During 24 h after onset (unknown)	Yes	Yes
N-methylhistamine (24-h urine)	During 24 h after onset (unknown)	Yes	Yes
N-methylimidazole acetic acid (24-h urine)	During 24 h after onset (unknown)	No	Unknown
Tryptase (serum/plasma)	15 min–3 h (60 min)	Yes	Yes
Chymase (plasma/serum)	0–24 h (unknown)	No	Unknown (correlates with tryptase)
Carboxypeptidase (plasma/serum)	0–8 h (unknown)	No	Unknown (does not correlate with tryptase)
Heparin (plasma)	Unknown	No	Yes
Newly generated over minutes			
Lipoxygenase product: LTE ₄ (24-h urine)	During 24 h after onset (unknown)	Yes (only in few commercial laboratories)	Yes
Cyclooxygenase product: PGD ₂ (24-h urine)	During 24 h after onset (unknown)	Yes (only in few commercial laboratories)	Yes
PAF (serum/plasma)	Unknown (very short life 3–13 min rapidly metabolized by PAF-AH)	No	Yes
Newly synthesized over hours			
IL-2 (serum/plasma)	0–10 h (100 min)	No	Yes
IL-4, IL-13 (serum/plasma)	Unknown	No	No
IL-5 (serum/plasma)	Unknown	No	No
IL-6 (serum/plasma)	0–10 h (100 min)	No	Yes
IL-10 (serum/plasma)	0–10 h (100 min)	No	Yes
TNF- α (serum/plasma)	Unknown	No	No
sTNFRI (serum/plasma)	0–10 h (100 min)	No	Yes
IFN-gamma (serum/plasma)	Unknown	No	No

IFN, interferon; IL, interleukin; LT, leukotriene; PAF, platelet-activating factor; PAF-AH, platelet-activating factor acetyl-hydrolase; PG, prostaglandin; sTNFRI, soluble tumour necrosis factor receptor I; TNF, tumour necrosis factor.

remain low (<11.4 $\mu\text{g/l}$) [25,28] or do not increase significantly above baseline [25].

Another limitation is that the method to measure tryptase is not an immediate procedure, so results will not be available during the acute episode of anaphylaxis.

In addition, functional tryptase is mostly a mature tetramer of β tryptase stabilized by proteoglycans; this type of tryptase is the one released during mast cell activation [8^o]. In fact, abnormal heparin formation alters mature tryptase contents of mast cells [8^o]. However, the available commercial methods measure basal immature α and β tryptase and mature tetramers of β tryptase. Therefore, the optimal method to assess mast cell activation would be to measure only tetrameric mature β tryptase. A recent study [29^o] describes a possible role of fragments of hFibrinogen (formed by proteolysis of tetramer forming tryptase) as

biomarkers of mast cell activation in anaphylaxis or mast cell disorders.

Furthermore, we must bear in mind that in anaphylactic reactions in which the main involved effector cell is not the mast cell, tryptase may not rise. For this reason, in Table 3, the origin of the main mediators in the main effector cells is summarized.

HISTAMINE

Plasma histamine peaks within 5–10 min of the onset of symptoms and declines to baseline within 30 min as a result of rapid metabolism by N-methyltransferase and diamine oxidase [19]. Therefore, blood samples need to be obtained at the onset of symptoms, which may only be possible in a small proportion of patients, given that reactions frequently occur outside a hospital, limiting its applicability. Histamine correlates with anaphylaxis severity [28].

Table 3. Origin of the main mediators in the main effector cells

Mediator	Mast cells	Basophils	Macrophages	Neutrophils
Preformed mediators				
Histamine	Yes	Yes	No	No
Tryptase	Yes	Yes (much lower than mast cells)	No	No
Chymase	Yes	No	No	No
Carboxypeptidase	Yes	No	No	No
Heparin	Yes	Yes	No	No
Newly generated over minutes				
Lipoxygenase product: LTE4	Yes	Yes	Yes	Yes
Cyclooxygenase product: PGD2	Yes	No	Yes	No
PAF	Yes	Yes	Yes	Yes
Newly synthesized over hours				
IL-1	No	No	Yes	No
IL-2	Yes	No	No	No
IL-4, IL-13	Yes	Yes	No	No
IL-5	Yes	Yes	No	No
IL-6	Yes	Yes	Yes	No
IL-10	Yes	No	No	No
IL-8	Yes	Yes	Yes	Yes
IL-17	No	No	No	Yes
IL-12	No	No	Yes	No
IL-23	No	No	Yes	No
IL-27	No	No	Yes	No
MCP-1	Yes	Yes	No	No
TNF- α	Yes	No	Yes	No
sTNFRI	Yes	No	Yes	No
IFN-gamma	Yes	No	No	No
RANTES	Yes	Yes	Yes	No

IFN, interferon; IL, interleukin; LT, leukotriene; MCP-1, monocyte chemotactic protein-1; PAF, platelet-activating factor; PAF-AH, platelet-activating factor acetylhydrolase; PG, prostaglandin; PG, prostaglandin; RANTES, regulated on activation normal T cell expressed and secreted; sTNFRI, soluble tumour necrosis factor receptor I; TNF, tumour necrosis factor.

HISTAMINE METABOLITES

The majority of released histamine is sequentially metabolized to N-methylhistamine and N-methylimidazole acetic acid by the N-methyltransferase and diamine oxidase [19]. These metabolites are longer-lived than histamine in the circulation and can be measured in plasma or in urine wherein they may appear within 30–60 min of the event. Normal levels do not exclude mast cell activation [30]; also, false positive results may occur due to ingestion of histamine-rich food [31].

CHYMASE

Like tryptase, chymase is a serine protease that is highly specific of MC_{TC}. Detection of tryptase or chymase provides information about mast cell distribution, numbers, activation status or

releasability, depending on the clinical context. Satomura *et al.* [32] established a method for determination of human chymase activity and applied this technique to measurements in serum and liver tissue. Nishio *et al.* [33] reported eight autopsy cases of anaphylaxis and they found elevated levels of chymase in all of them and in only two of the 104 control autopsies, with positive correlation with tryptase [33]. The group of Zhou *et al.* [34] also reported a study of 181 patients with anaphylaxis with a greater level of chymase in serum than controls; chymase remained high at least 24 h after onset of the reaction.

CARBOXYPEPTIDASE

Zhou *et al.* [35] also investigated the role of carboxypeptidase during anaphylaxis in 181 patients and

they found that carboxypeptidase levels in serum or plasma collected within 8 h of the onset of an allergic reaction were significantly greater than in those of either of the control groups. There was no correlation with tryptase levels. However, in 70% of patients with negative tryptase, levels of carboxypeptidase were elevated.

Chymase and carboxipeptidase are not expressed by basophils, justifying further research.

HEPARIN

Recently, Sala-Cunill *et al.* [36[■]] have reported the role of heparin in activating the plasma contact system. They describe that IgE/antigen-stimulated mast cells release heparin locally, which induces bradykinin formation in a FXII-dependent manner in mice and also in human anaphylaxis. During anaphylaxis, patients presented a high-molecular-weight kinyogen cleavage with bradykinin formation and high levels of anti-Xa (an indirect measure of plasma heparin) compared with controls. The study also suggests that activated mast cells release heparin into circulation. This heparin, due to its negative charge, is a potent FXII contact activator in humans, inducing contact system-mediated bradykinin formation in plasma. This bradykinin will increase the vascular permeability. They also found a statistically significant correlation between the severity and bradykinin levels [36[■]].

CYSTEINYL LEUKOTRIENES AND PROSTAGLANDINS

Leukotriene C₄ (LTC₄) is the major 5-lipoxygenase product produced by activated mast cells and basophils. Platelets, eosinophils, endothelial cells and vascular smooth muscle cells also produce LTC₄. It is rapidly metabolized to LTD₄ and then to LTE₄, which can be measured in urine [37].

Prostaglandin D₂ (PGD₂) is the main cyclooxygenase product produced by activated MCs, but not by activated basophils. Like histamine and LTC₄, clearance from the systemic circulation is rapid, and elevated serum levels are difficult to detect. Its metabolite (11-beta-PGF₂-alpha) can be measured in urine and is more sensitive than N-methylhistamine [38]. However, measurement of PGD₂ cannot be used in patients receiving cyclooxygenase inhibitors.

Ono *et al.* [37] assessed the concentrations of various inflammatory mediators in urine samples from 32 patients during anaphylaxis and in clinical remission. They found a significant increase in urinary LTE₄ and of 9 α , 11b-prostaglandin F₂ concentrations during anaphylaxis compared with controls

and baseline [37], with a positive correlation between both markers.

PLATELET-ACTIVATING FACTOR

PAF is a proinflammatory phospholipid synthesized and secreted by mast cells, monocytes and fixed tissue macrophages [10]. Circulating levels of PAF are, in part, controlled by the activity of PAF acetylhydrolase (PAF-AH), which is the enzyme that degrades PAF [39]. Transduction of biologic signals after the binding of PAF to its receptor on platelets, monocytes, macrophages and neutrophils results in many of the manifestations of anaphylaxis [40]. Because of its rapid inactivation by PAF-AH, it has a short elimination half-life, ranging from 3 to 13 min [41].

PAF is an extremely potent biological mediator that is active at concentrations as low as 10–12 mol/l. In 2008, Vadas *et al.* [41] investigated serum PAF and PAF-AH activity in 41 patients with anaphylaxis and in 23 controls. They found that serum PAF levels were directly correlated and serum PAF-AH activity was inversely correlated with the severity of anaphylaxis [41]. They concluded that PAF correlates better with the severity of acute allergic reactions than either histamine or tryptase. These data suggest an important contributory role for PAF in morbidity and mortality in human anaphylaxis [42]. The same group also demonstrated low levels of PAF-AH in patients with fatal peanut anaphylaxis [43].

Recently, Pravettoni *et al.* [44[■]] examined the basal PAF-AH levels in a large population of patients with hymenoptera venom allergy (HVA) experiencing systemic reactions. The only factor that significantly correlated with the basal PAF-AH level was anaphylaxis severity. The level indicates the genetic background for an individual and its predictability for anaphylaxis severity in the future [44[■]].

CYTOKINES

Stone *et al.* [28] analysed the peak concentrations of different mediators including histamine, tryptase, cytokines [IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, interferon (IFN)-gamma and tumour necrosis factor (TNF)-alpha and TNFRI] and chemokines in a prospective study. All these mediators were higher in anaphylactic patients than in the control group. However, only tryptase, histamine, IL-2, IL-6, IL-10 and TNFRI correlated with hypotension [28]. The best timing to measure peaks for each mediator was within 30 min after reaction onset for histamine and 30–60 min for tryptase, with tryptase declining more slowly than histamine. The peaks in IL-6, IL-10 and TNFRI were relatively delayed (around 100 min)

and sustained for as long as 10h. IL-2 concentrations followed a similar pattern [28].

More recently Brown *et al.* [45] defined the clinical patterns of anaphylaxis and relationships between mediators and severity. They also found that tryptase, histamine, IL-6, IL-10 and TFNRI were associated with severity and with delayed deteriorations. In addition, low PAF-AH activity was associated with severe reactions as described before [41].

More recently, but only in murine models, several novel mediators that may propagate and/or regulate the immune response have been identified. These include sphingosine-1-phosphate, galectin-9, IL-33 and nitric oxide [46–49].

CONCLUSION

It is now clear that anaphylaxis involves the activation of multiple immune pathways, cell types and a wide range of mediators, depending on different factors such as route of exposure or specific trigger. Subsequently, measuring only one mediator may not be sufficient to diagnose anaphylaxis and also targeting only one pathway may not prevent or treat anaphylactic responses. This could partly explain the current lack of optimal biomarkers to confirm the diagnosis of anaphylaxis. Identification of key pathways that contribute to the amplification of the allergic signal early during anaphylaxis and primary interventions to prevent these catastrophic reactions should be a future research priority.

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- of special interest
- of outstanding interest

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