




New Biomarkers in Anaphylaxis (Beyond Tryptase)

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Abstract

Purpose of Review The purpose of this review is to provide a better understanding of anaphylaxis pathophysiology and describe the underlying mechanisms, effector cells, and the potential biomarkers involved depending on the anaphylaxis endotypes.

Recent Findings New insight into the potential relevance of pathways others than IgE-dependent anaphylaxis has been unraveled, as well as other biomarkers than tryptase, such as the role of platelet activation factor, basogranulin, dipeptidyl peptidase I, CCL-2, and other cytokines.

Summary Gaining knowledge of all the mediators and cellular activation/communication pathways involved in each endotype of anaphylaxis will allow the application of precision medicine in patients with anaphylactic reactions, providing insights to the most appropriate approach in each case and helping to stratify severity and risk prediction.

Introduction

Anaphylaxis is a severe, multisystem syndrome that is rapid in onset and potentially lethal, requiring immediate medical intervention. However, the diagnosis of anaphylaxis is still based on clinical symptoms after exposure to a potential allergen or event, given that no biomarker allows an unequivocal diagnosis of anaphylaxis [1, 2•]. Therefore, anaphylaxis remains under-recognized, undertreated, and poorly understood. The current gold standard laboratory test involves the measurement of serum total mast cell tryptase (MCT) during an acute phase followed by a baseline measurement at least 24 h after the reaction. However, even when sampling of tryptase is timely, an increase is not detectable in all cases [1, 2•].

Phenotypes of anaphylaxis are defined by clinical presentation and endotypes are based on cellular and molecular mechanisms involved during an anaphylactic reaction [1, 2•]. The activation of different pathways during an anaphylaxis will depend on which receptors are activated in the underlying effector cells and which mechanism and mediators are involved in the reaction. This review summarizes the most recent knowledge of how different effector cells and their mediators are involved in anaphylaxis depending on the mechanism providing a better understanding of the pathophysiology of anaphylaxis and explores and discusses the role for new biomarkers of anaphylaxis beyond tryptase.

Endotypes and effector cells involved in anaphylaxis

The type of receptor activated in the different effector cells during an anaphylactic reaction will influence which mediators (tryptase, interleukin [IL]-6, ...) are released and, subsequently, which can be potential diagnostic biomarkers [3••]. Depending on these cellular and molecular mechanisms, anaphylaxis is divided in different endotypes, summarized in Table 1.

The major molecular mechanism underlying anaphylaxis is the classic allergic IgE-mediated reaction involving mast cells (MC) and basophils. Both cell types express the high-affinity IgE receptor (FcεRI) on their surface and are considered the main effector cells in this pathologic event [4]. However, a considerable percentage of subjects do not show evidence of IgE-dependent immune activation; therefore, other effector cells and pathways must be involved in anaphylaxis. Nowadays, there is enough evidence to corroborate that IgG antibodies can induce anaphylaxis by binding to their different receptors (FcγR) [5••]. Such receptors are found in MC, basophils, neutrophils, monocytes, and macrophages conforming the major cellular types activated by this alternative pathway [3••]. In fact, some studies have shown that human IgG receptors are capable of activating macrophages and neutrophils to secrete platelet-activating factor (PAF), and then PAF can activate mast cells *in vitro*, reinforcing the role of this mediator in human anaphylaxis as described below [6]. Moreover, the amount of antibody and antigen seems to be crucial in determining IgG- or IgE-dependent anaphylaxis. Thus, to induce a similar response in IgG-dependent anaphylaxis, a higher dose of antigen is required compared to IgE-dependent [7]. Complement-mediated anaphylaxis has also been described in human anaphylaxis [8]. C3a and C5a, known as anaphylatoxins, are potent inflammatory mediators generated upon activation of the complement cascades. Some substances, such as peanuts, have been described to have the ability to rapidly activate complement,

Table 1 Main effector cells, receptors, and endotype involved in anaphylaxis (3–14, 19, 21, 23, 27–31, 33, 35–38, 40, 42, 43, 46, 47, 49, 80, 83)

	Receptor	Endotype
Mast cell		
Activators	FceRI	IgE mediated
	FceRII (CD23)	IgE mediated
	FcγRIIa	IgG mediated
	Mrgprs	IgE independent
	Trk	Non-immunological
	Kit/CD117 (SCFR)	Non-immunological
	μ-δ-opioid receptors	Non-immunological
	TLR 1–9	IgE independent
	NTAL	Unknown
	C3aR, CD88/C5aR	Complement mediated
	HR 1–2–3–4	All
	PAFR	All
	CD200 R1	Non-immunological
	Neurokinin 1-receptor	Non-immunological
	IL-33R, TSLPR	Unknown
	IL-4R	Unknown
	IL1R	Cytokine release syndrome
	IL-6R soluble	Cytokine release syndrome
Basophil		
Activators	FceRI	IgE mediated
	FcγRIIa (CD32)	IgG mediated
	Mrgprs	IgE independent
	Trk	Non-immunological
	TLR	IgE independent
	C3aR, CD88/C5aR	Complement mediated
	CD35/C3b/C4b/CR1 CD11b/CD18/CR3, C3bR, CD11c/CR4	Complement mediated
	HR 1–2–4	All
	PAFR	All
	IL-33R, IL-25, TSLPR	Unknown

Table 1 (continued)

	Receptor	Endotype
Eosinophil Activators	FcεRI	IgE mediated
	FcεRII (CD23)	IgE mediated
	FcγRIIa (CD32)	IgG mediated
	Trk	Non-immunological
	TLR	IgE independent
	C3aR, CD88/C5aR	Complement mediated
	CD35/C3b/C4b/CR1 CD11b/CD18/CR3, C3bR	Complement mediated
	HR 1-2-3-4	All
	PAFR	All
	IL-33R, IL-25, TSLPR	Unknown
	IL-1R	Cytokine release syndrome
	IL-6R soluble	Cytokine release syndrome
	TNFR	Cytokine release syndrome
Neutrophil Activators	FcεRI	IgE mediated
	FcεRII (CD23)	Proteases
	FcγR (I-IV)	IgG mediated
	Trk	Non-immunological
	TLR	IgE independent
	C3aR, CD88/C5aR	Complement mediated
	CD35/C3b/C4b/CR1 CD11b/CD18/CR3	Complement mediated
	HR 1-2-4	All
	PAFR	All
	G-CSFR, GM-CSFR	All
	IL1R	Cytokine release syndrome
	IL-6R soluble	Cytokine release syndrome
	TNFR	Cytokine release syndrome
Monocyte/macrophage Activators	FcεRI	IgE mediated
	FcγR (I-III)	IgG mediated
	Mrgprs	IgE independent
	Tyr	Non-immunological
	TLR	IgE-independent
	C3aR, CD88/C5aR	Complement activation
	CD11b/CD18/CR3, CD35/C3b/C4b/CR1	Complement activation
	HR 1-2-4	All
	IL-6R soluble	Cytokine release syndrome
	TNFR	Cytokine release syndrome

Table 1 (continued)

	Receptor	Endotype
Platelet		
Activators	FcεRI	IgE mediated
	FcεRII (CD23)	Proteases
	FcγRIIa	IgG mediated
	TLR	IgE independent
	C3b/CD46	Complement mediated
	C1qR	Complement mediated
		Contact system mediated
	IL1R	Cytokine release syndrome
	IL-6R soluble	Cytokine release syndrome
	TNFR	Cytokine release syndrome
	Epithelium	
Vasodilation, increased permeability	HR 1–2	All
	PAFR	All
	PAR2 (tryptase-R)	All
	CysLTR 1–2	All
	C3aR, CD88/C5aR	Complement activation
	Bradykinin 1–2 R	Contact system activation
	EP1-2–4, DP	All
	IL-4Ra	Unknown
	IL1R	Cytokine release syndrome
	IL-6R soluble	Cytokine release syndrome
	TNFR	Cytokine release syndrome

FcεRI, high affinity IgE receptor; *FcεRII (CD23)*, low affinity IgE receptor; *FcγRIIa*, IgG receptor; *Mrgprs*, Mas-related G protein-coupled receptor; *Trk*, tyrosine kinase receptor; *SCFR*, stem cell factor receptor; *TLR*, Toll-like receptor; *NTAL*, non-T cell activation linker; *HR*, histamine receptor; *TNFR*, tumor necrosis factor receptor; *PAFR*, plasmin activator factor receptor; *TSLPR*, thymic stromal lymphopoietin receptor; *G-CSFR*, granulocyte colony stimulating factor receptor; *GM-CSFR*, granulocyte–macrophage colony-stimulating factor receptor; *PAR*, protease activated receptor; *CysLTR*, cysteinyl leukotriene receptor; *EP*, E prostanoid receptor; *DP*, D prostaglandin receptor

with production of large amounts of anaphylatoxins that stimulates macrophages, basophils, and MC which contribute to the induction of anaphylaxis [9, 10]. In addition, the contact and coagulation systems can be activated in anaphylaxis through immunological and non-immunological mechanisms [11].

Different non-immunologic activating paths have been described in anaphylaxis. Some drugs (e.g., opioids, neuromuscular blockers, quinolones) are also capable of activating MC and basophils, triggering degranulation, through the Mas-related G-protein coupled receptor member X2 (MRGPRX2) [12, 13]. This is a highly relevant pathway independent of specific immunoglobulins. External factors such as physical exercise, exposure to cold, or ultraviolet radiation may also act as elicitors of MC [14, 15••, 16]. Another endotype discovered due to the increasingly common use of monoclonal antibodies and chemotherapeutic agents is cytokine release reaction. These reactions are suspected to be mediated by tumor necrosis factor alpha (TNFα), IL-1β and IL-6 produced by MC, monocytes, and T cells. In some patients, the endotype could be mixed due to co-occurrence of IgE-mediated reactions and cytokine release reactions [4].

Considering all the above, anaphylaxis endotypes can be divided mainly in two different groups, immune-mediated anaphylaxis (IgE-dependent and non-IgE-dependent such as IgG-dependent, complement system, contact system, cytokine release reactions) and non-immunological anaphylaxis.

Mast cell

MC are hematopoietic cells that reside in different tissues (such as connective tissue, blood, skin, among others) and have an important role in inflammation. MC are the key effector cells in anaphylaxis [16, 17]. They are involved in IgE-mediated reactions (immediate hypersensitive reactions), due to cross-linking by allergens of specific IgE (sIgE) attached to its high-affinity receptor (FCεRI) expressed predominantly by MC and basophils [18]. This interaction, allergen-sIgE-MC, results in intracellular signaling that triggers the release of MC mediators. There are two mayor types of MC, MC (T) that are characterized by a large content in tryptase and MC (TC) which produce both tryptase and chymase [19]. Characteristically, MC express an array of different receptors, the activation of which may induce activation through diverse pathways, as explained above. Figure 1 outlines the different MC activating receptors and mediators.

Basophil

Basophils are the largest and least common granular immune cells, representing about 0.5 to 1% of circulating leukocytes. Unlike MC, they are blood-circulating leukocytes and not tissue-resident cells [20, 21] Recent studies suggest that basophils activate complementary to MC and they play a key role in anaphylaxis, especially in food-induced anaphylaxis [22, 23]. Basophils share many mediators and receptors with MC [24] and are more commonly used to perform laboratory allergen-specific activation tests (BAT), since they are easy to sample from whole blood. BAT has been demonstrated useful to confirm IgE-mediated allergy and also to monitor allergen immunotherapy, in order to differentiate short-term desensitization versus sustained unresponsiveness to the allergen [25, 26].

Neutrophil, eosinophil, monocyte/macrophage, and platelet

The most recent studies on anaphylaxis have highlighted the role of other immune effector cells such as monocytes, macrophages, neutrophils, eosinophils, and platelets. Most of this evidence is based on murine models, so knowledge about their involvement in human anaphylaxis still remains scarce [27].

The implication of both monocytes and macrophages has been demonstrated in passive and active systemic anaphylaxis. They have been more

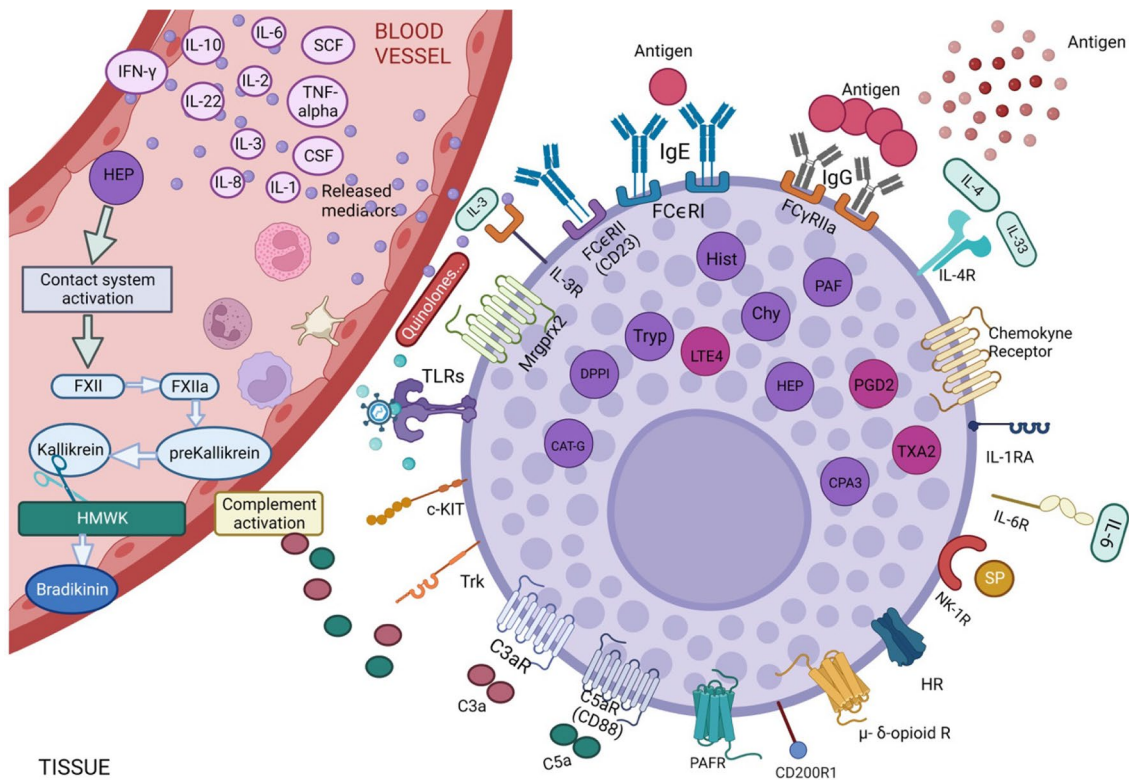


Fig. 1 The different MC activation receptors and mediators. FcεRI, high affinity IgE receptor; FcεRII (CD23), low affinity IgE receptor; FcγRIIa, IgG receptor; MrgprX2, Mas-related G protein-coupled receptor; Trk, tyrosine kinase receptor; TLR, Toll-like receptor; HR, histamine receptor; PAFR, plasmin activator factor receptor; NK-1R, neurokinin receptor 1; Tryp, tryptase; Hist, histamine; PAF, platelet activator factor; Chy, chymase; CPA3, carboxypeptidase A3; DPPI, dipeptidyl peptidase I/cathepsin C; CAT-G, cathepsin G; LTE4, lipoxygenase product E4; PGD2, prostaglandin D2; TXA2, thromboxane A2; HEP, heparin; FXII, factor XII; FXIIa, activated factor XII; CSF, stem cell factor; TNF- α , necrosis tumoral factor alpha. This image is created with BioRender.com

related to IgG-dependent and cytokine release reaction endotypes [27–29]. However, especially neutrophils have gained much relevance as key cellular player eliciting non-IgE-dependent anaphylactic reactions [4, 30]. Evidence in patients has shown elevated circulating serum levels of neutrophil elastase and myeloperoxidase, major mediators stored in their granules. These results supported the existence of a neutrophil-associated IgG molecular mechanism associated with drug-induced anaphylaxis [31–33]. As represented in Table 1, neutrophils not only express FcγR, but also FcεRI and MRGPRX2.

MC and eosinophils share several receptors and mediators (Tables 1 and 2), so suspecting their involvement in anaphylaxis seems reasonable. Accumulation of eosinophils has been detected in passive cutaneous anaphylactic reactions in guinea pigs, as well as in spleens and coronary arteries from anaphylactic human cadavers [34, 35]. And lastly, but by no means less importantly, there is evidence of platelet contribution in anaphylactic reactions. The release of important mediators (Table 2) has been observed in both IgE- and IgG-dependent pathways [6, 36–38].

Taken together, this data indicate that many myeloid cells have a role in the physiopathology of anaphylaxis.

Endothelium

The endothelium consists of a single and extensive layer of endothelial cells (ECs). Molecularly, stabilization of the endothelial barrier depends on a series of connections, such as tight junctions (TJ) and adherent junctions (AJ). A dysfunctional endothelium is the cause of important cardiovascular diseases such as thrombosis, atherosclerosis, or hypertension [39]. A damaged endothelium has been observed in patients with mastocytosis [40] and, in acute inflammatory situations as in COVID-19 disease, ECs contribute as effector cells to the cytokine releases syndrome [41]. Therefore, endothelium is an important organ-cell in anaphylaxis playing a role not only in the control of fluids and the vascular tone but also as an activation surface for the coagulation, contact system, and complement [11, 42, 43]. ECs release relevant anaphylactic mediators such as nitric oxide (NO), together with other molecules (presumably cytokines or interleukins) which also released, contribute to the pool of mediators in the reaction [3••]. Specifically, anaphylactic shock depending on endothelial Gq/G11 has been characterized in mice [43]. However, the exact contribution of these cells during anaphylaxis is still not fully understood.

Mediators release in anaphylaxis

During anaphylaxis there is a rapid release of mediators from different sources, but most important, from MC and basophils. These mediators are proteases, sulfated polysaccharides, cytokines, chemokines, vasoactive agents (histamine, bradykinin) and proinflammatory lipid mediators, among others [44]. They are usually divided into three classes: preformed mediators (that are stored in cytoplasmic granules), newly generated in minutes (proinflammatory lipid mediators), and newly generated over hours (growth factors, cytokines, and chemokines). Table 2 summarizes the main mediators, their major cell sources, best sampling time, and the availability of commercial assays.

Preformed mediators

Preformed mediators are stored in cytoplasmic granules of the effector cells (mainly MC and basophils) and include proteases (tryptase, chymase), highly sulfated polysaccharides (heparin and other proteoglycans), and histamine [45].

Table 2 Summary of anaphylaxis mediators, cell source, testing characteristics, and evidence of their potential use as biomarkers in anaphylaxis (1–7, 9–22, 24, 27–40, 42–90)

Mediator	Source	Recommended sampling time	Methods available for routine assessment	Correlation with severe anaphylaxis	Half-life
Preformed					
Histamine (plasma, urine)	MC, BS NT	0–30 min	Yes	Yes	< 15 min
Histamine (24-h urine)	MC, BS, NT	0–24 h	Yes	Yes	UNK (0–24 h)
N-m-histamine (plasma, 24-h urine)	MC, BS, NT	0–24 h	Yes	Yes	UNK (0–24 h)
N-m-imidazole acetic acid (24-h urine)	MC, BS, NT	0–24 h	Yes	UNK	UNK (0–24 h)
Tryptase (serum/plasma)	MC, BS	15 min–3 h	Yes	Yes	~2 h
Chymase (serum)	MC, BS	0–24 h	No	Yes	>24 h
Carboxypeptidase A3 (serum/plasma)	MC, BS	0–8 h	No	UNK	> tryptase (2–8 h)
Basogranulin (serum)	BS	UNK	No	No	UNK
Heparin (plasma)	MC, BS	UNK	No	Yes	UNK
DPPII/cathepsin C (serum)	MC, BS	UNK	No	UNK	UNK (similar to histamine)
Cathepsin G (serum)	MC, BS, EOS, NT	UNK	No	UNK	UNK
Newly generated over minutes					
Bradykinin (plasma)	Plasma	UNK	No	Yes	< 1 min
FXII, PK, HK, thrombin (plasma)	Plasma	UNK	FXII, thrombin	UNK	UNK
LTE4 (24-h urine, plasma)	MC, BS, EOS, NT, MAC	0–24 h	No	Yes	7 min
LTB4, LTC4, LTD4 (24-h urine)	MC, BS, NT	0–24 h	No	UNK	< 7 min
PGD2 (serum)	MC, EOS, NT, MN, PT	0–24 h	No	Yes	30 min
9 α ,11 β -PGF2 (serum)	MC, PT	UNK (5–15 min)	No	UNK	UNK
2,3-dinor-11 β -PGF2 α (24-h urine)	MC, PT	UNK (0–24 h)	No	UNK	UNK
TXA2 (serum/plasma, urine)	MC, EOS, PT	UNK	No	UNK	30 s
TXB2 (serum/plasma, urine)	MC	UNK	No	UNK	60 min
PAF* (plasma)	MC, BS, EOS, NT, MAC, MN, PT, EC	UNK	No	Yes	< 5 min
PAF-AH (serum)	MC, MAC, PT plasma	UNK	No	Yes	30 min
Nitric oxide	EC	UNK	No	UNK	UNK

Table 2 (continued)

Mediator	Source	Recommended sampling time	Methods available for routine assessment	Correlation with severe anaphylaxis	Half-life
C3a, C5a (plasma)	Plasma	UNK	No	No	UNK
TNF-α* (serum/plasma) Newly generated over hours	MC, MAS, NT, MAC	UNK	Yes	UNK	30 min
Stem cell factor (serum/plasma)	MC	UNK	No	Yes**	UNK
TGF-β (serum/plasma)	MC, EOS	UNK	Yes	UNK, potential**	2–3 min active 90 min latent**
CXCL4/PF4, PDGF	PT	UNK	No	UNK	<3 min
IL-1b (serum/plasma)	MC, NT	0–15 min	No	UNK	19 min**
IL-2, IL-22 (serum/plasma)	MC	0–10 h	No	IL-2 yes	UNK
IL-4/IL-13 (serum/plasma)	MC, BS, EOS	UNK (0–24 h)	No	IL-4 yes**	35–90 min
IL-10 (serum/plasma)	MC, EOS	UNK (0–24 h)	Yes	Yes	1–3.5 h
IL-6 (serum/plasma)	MC, BS, NT, MAC	UNK (0–24 h)	Yes	Yes	2–6 h
IL-8 (serum/plasma)	MC, EOS, NT	UNK (<3 h)	No	No	<4 h
CCL2/MCP-1 (serum/plasma)	MC, NT, MAC, MN, EC	UNK (0–30 min)	No	UNK, potential	30 min
CCL5/RANTES (serum/plasma)	MC, EOS, MN	UNK	No	UNK	14–18 h
MPO (serum/plasma)	NT, MN	UNK (<5 h)	No	Yes	UNK 4–5 h**
Other possible biomarkers (source cells)	Chondroitin sulfate (MC, BS), elastase* (NT), TSLP (MC, EC, SMC), IL-31 (MC, BS, EOS, MAC, LT), IL-33 (MC, BS), IL-3/IL-5/IL-16/IL-18 (MC, EOS), IL-17 (NT), IL-23/IL-27/FGF (MAC), G-CSF (MC, NT, MAC, MN, EC), GM-CSF (MC, BS, EC), M-CSF (MC, MN, EC), VEGF (MC, MAC, PT), S1P (MC, PT, EC, ERY), IFN-γ (NT, LT, NK), endothelin-1 (MC, MAC, MN, EC, SMC), prostacyclin (MC, PT, EC, SMC), plasminogen activator (MC), MBP (EOS)				

MC, mast cell; BS, basophil; NT, neutrophil; PT, platelet; EOS, eosinophil; MAC, macrophage; MN, monocyte; EC, endothelial cell; SMC, smooth muscle cell; ERY, erythrocyte; LT, T lymphocyte; NK, natural killer cell; M-m, N-methyl; DPPI, dipeptidyl peptidase I; FXII, coagulation factor XII; PK, prekallikrein; HK, high molecular weight kininogen; PAF, plasmin activator factor; PAF-AH, acetylhydrolase; LIE4, E4 leukotriene; LTB4, B4 leukotriene; LTC4, C4 leukotriene; LTD4, D4 leukotriene; PGD2, D2 prostaglandin; TXA2, A2 thromboxane; TXB2, B2 thromboxane; TGF-β, tumor growth factor beta; TNF-α, tumor necrosis factor alpha; CXCL4/PF4, platelet factor 4; PDGF, platelet-derived growth factor; TSLP, thymic stromal lymphopoietin; FGF, fibroblast growth factor; G-CSFR, granulocyte colony stimulating factor receptor; GM-CSFR, granulocyte-macrophage colony-stimulating factor receptor; M-CSF, VEGF CCL2/MCP-1 CCL5/RANTES S1P; MPO, myeloperoxidase; MBP, major basic protein; IFN-γ, interferon gamma

* Also performed, but mainly newly generated

** Studies performed in mammals, not in humans

Histamine

Plasma histamine is an amine secreted by MC and basophils with vasoactive and pro-inflammatory functions. It is synthesized from the amino acid histidine and catalyzed by L-histidine decarboxylase. Histamine is stored in cytoplasmic granules and can be released when immunological or non-immunological activation occurs. There is evidence that neutrophils can also synthesize and release histamine under specific circumstances [46]. When MC/basophils are activated, histamine is released and it acts through its G-protein receptors (H1, H2, H3, and H4), causing arteriolar vasodilation, increased capillary permeability, bronchoconstriction, itching, swelling, rhinorrhea, urticaria, and rhinitis, among others [47]. The peak of histamine is 5–10 min from the onset of the reaction and is rapidly metabolized into methyl histamine by the N-methyltransferase or into imidazole acetaldehyde by diamine oxidase, and then secreted in urine [48]. In daily practice, histamine is not a good diagnostic biomarker for anaphylaxis due to its short half-life [4, 49].

Histamine metabolites

Methyl histamine and imidazole acetaldehyde had longer half-life compared to histamine and can be measured in urine or plasma after 30–60 min of the onset of the reaction [48]. Normal levels do not exclude a hypersensitivity reaction. Some false positive results may occur due to ingestion of foods with high concentrations of histamine (in example, in scombroidosis), endogenous production by other type of cells such as neurons or enterochromaffin-like cells in stomach, or by certain bacteria [24].

Tryptase

Tryptase is a neutral serine protease highly specific of MC, stored in large amounts in cytoplasmic granules. It is currently used as a diagnostic biomarker in anaphylaxis. Also, basophils produce tryptase but in much lower amounts. Tryptase provides information about MC activation, distribution, and number so it is useful for diagnosis and follow-up of mast cell diseases [50, 51].

There are two biologically important forms of tryptase, alpha-tryptase and beta-tryptase. The alpha-protryptase is present in serum as an inactive proenzyme and is secreted constitutively. Beta-tryptase, on the other hand, is the functional tryptase, a mature tetramer stabilized by proteoglycans, stored predominantly in MC secretory granules and released by active MC. Tryptase acts binding to protease activated receptor type 2 (PAR-2, present in gastrointestinal, cardiovascular, neurons, among others). It is responsible of cleaving extracellular substrates, such as calcitonin gene-related peptide, kininogens, fibronectin, and vasoactive

intestinal peptide; also, it is a potent growth factor for EC, airway smooth muscle cells, and fibroblasts [45, 52]. It also has a chemotactic effect on neutrophils and eosinophils via IL-8 [53]. During anaphylaxis, tryptase is responsible for vascular vasodilation (activating the contact system to generate bradykinin) and bronchial hyperresponsiveness [49].

Basal tryptase in healthy individuals ranges from 0 to 11.4 ng/mL. Currently, it is accepted that levels of 120% of the baseline plus 2 ng/mL ($1.2 \times$ baseline tryptase + 2 ng/mL) is a significant increase, indicating MC activation (54, 55). In MC disorders, a basal tryptase > 20 ng/mL is considered as a minor criteria of mastocytosis [51].

During active MC degranulation in allergic reactions, β -tryptase is released. Levels of tryptase are increased from 15 min to 3 h after the anaphylaxis onset [54, 56]. Sequential measurement of serum tryptase during anaphylaxis and basal serum levels increases sensitivity and specific of the diagnostic. Higher peak/baseline tryptase correlates with more severe symptoms [57]. Nevertheless, it is important to take into account that an increase is not detectable in all cases, for example, in food-induced anaphylaxis where tryptase does not increase as in drug-induced reactions [56, 58], but may still be useful for diagnosis. Current commercial methods measure immature alpha and beta tryptase, but as a diagnostic marker of anaphylaxis, it would be optimal to measure beta-tryptase alone, since this is the form released by MC.

Chymase

Chymase is a serine endopeptidase also found in MC (TC). It is usually released during MC/basophil activation, and its concentration in serum has been seen to be stable. There are no commercialized kits to measure chymase, so is not used routinely in anaphylactic reactions [59]. Chymase participates in local arterial pressure regulation due to the hydrolysis of angiotensin I into angiotensin II [60]; it also activates endothelin-1 and 2, leading to the production of metalloproteinases and cytokines [24]. It has been reported alternative methods to evaluate indirectly Chymase via alpha-2-macroglobulin, but is not systematically used [61]. Postmortem chymase concentrations in cases of fatal anaphylaxis have been shown to correlate with levels of tryptase [62].

Carboxypeptidase A3

Carboxypeptidase A3 is another metalloproteinase contained in MC and basophil granules [63]. Zhou et al. [64] measured levels of carboxypeptidase A3 in cases of anaphylaxis, systemic mastocytosis, and control groups. Significantly elevated levels were found 8 h after the onset of an allergic reaction compared to the control cohort. There are no available commercial methods for its quantification in daily practice.

Basogranulin

Basogranulin is a basophil granule protein that is released in parallel with histamine. It shares receptors and pathways with histamine [65]. Nowadays, there are no available methods for its measurement [66].

Heparin

Highly sulfated proteoglycan heparin is preformed in MC (predominantly) and also in basophil granules. It is stored in its active form with a negative charge. It has been reported that heparin has an important role in activating the plasma contact system [11, 67]. Due to its negative charge, it is capable of activating the FXII-dependent pathway and cleaving high molecular weight kininogen into bradykinin, leading to increased vascular permeability [11].

Dipeptidyl peptidase I (DPPI)/cathepsin C

Dipeptidyl peptidase I (DPPI)/cathepsin C is a member of the papain family proteases that is expressed in numerous cell types, including both MC and basophils. Levels of chymase and DDPI have been shown to increase and correlated during anaphylaxis, but do not correlate with tryptase [68, 69]. Its activity outside of cells is limited. There are no available measurement methods for its quantification currently.

Cathepsin G

Cathepsin G is a protease related to chymase and has similar functions (cleavage of angiotensin I to angiotensin II) [69]. It is found in MC granules. There are no available techniques to measure it.

Newly generated over minutes

Newly generated mediators are generated over minutes after the onset of stimuli by the different types of cells involved in the reactions.

Bradykinin (BK)

Is a nonapeptide from the kinin family with vasoactive functions that has been demonstrated to be released during anaphylaxis by activation of the contact system [11, 67]. When the contact system is activated by a negative charge (for instance heparin that is released in anaphylaxis), FXII is activated to FXIIa, which cleaves prekallikrein to kallikrein, which subsequently hydrolyses the high molecular weight kininogen to release BK. BK has a very short half-life, less than 1 min, due to its fast metabolism by the specific enzymes (angiotensin

converting enzyme, also known as kininase II), dipeptidyl peptidase IV, carboxypeptidase N also known as kininase I, and neutral endopeptidase [70]. BK acts through its specific receptors, B1 and B2, inducing an increase of vascular permeability and plasma extravasation [71]. Currently, it is not possible to measure BK in daily practice, due to its short half-life, so indirect methods are used to assess it in research setting.

Lipid mediators

Lipid mediators are synthesized de novo in activated MC and basophils. They derive from arachidonic acid and have vasoactive and pro-inflammatory functions.

Lipoxygenase products: leukotriene E4

Leukotriene E4 (LTE4) is produced by activated MC, neutrophils, eosinophils, and macrophages. It is derived from the precursor LTA4, converted to LTD4 and finally LTE4. LTE4 is the most stable metabolite of the lipoxygenase products and binds to three different receptors (CysLTR1, CysLTR2, CysLTR3). Its activation causes airway constriction, smooth muscle contraction, and increased vascular permeability [24]. LTE4 can be measured in urine, but due to their rapid metabolism, LTC4 and LTD4 cannot be measured [72].

Cyclooxygenase products: prostaglandins and thromboxanes

Generation of metabolites of arachidonic acid (thromboxane A2, thromboxane B2, prostaglandin (PG) D2, PGE2, PGF2) in anaphylaxis has been demonstrated in several studies with animals and humans [73, 74]. PGD2 is the main cyclooxygenase product formed in activated MC, also produced by neutrophils, monocytes, macrophages, and platelets, but not by basophils. PGD2 binds to its receptors DP1 and/or DP2 and increases vascular permeability and induces bronchoconstriction. PGD2 has a rapid degradation to more stable metabolites such D-prostaglandin (PGD), F-prostaglandin (PGF), and J-prostaglandin (PGJ), which are excreted in urine [75]. Nassiri et al. [76] suggest that serum levels of 9 α ,11 β -PGF2 could be useful biomarkers of anaphylaxis. The urinary metabolite 2,3-dinor-11 β -PGF2 α can be measured in urine [75].

Platelet-activating factor (PAF)

PAF is a potent phospholipid derived mediator produced by activated MC, endothelial cells, polymorphonuclear (PMN) leukocytes, eosinophils,

macrophages, monocytes, and platelets. It is rapidly metabolized into its inactive metabolite lyso-PAF (by the enzyme PAF acetylhydrolase—PAF-AH). During anaphylaxis, PAF binds to its receptor on monocytes, platelets, macrophages, and neutrophil. It induces a potent bronchoconstriction and vasodilation with edema. Also is involved in platelet aggregation, activation of eosinophils, and PMN-leucocytes. Elevated levels of PAF correlate with the severity of anaphylaxis [77, 78]. Also, there is an inverse correlation between PAF and PAF-AH activity. Vadas et al. (79) showed that PAF correlates better to MC anaphylaxis compared to histamine and tryptase. PAF is common in both, IgE and IgG anaphylaxis, due to that it is released by all myeloid cell subsets. In mouse models, it had been demonstrated a synergistic effect with histamine [27]. One of the challenges of measuring PAF and PAF-AH in a daily practice is its short half-life and the transport precautions it requires.

C3a and C5a

Activation of complement by immune complexes can lead to the generation of anaphylatoxins (AT), such as C3a and C5a, which can then bind to complement receptors on MC. C3a and C5a regulate vasodilation, increase the permeability of small blood vessels, and induce contraction of smooth muscles. Basophils and MC react upon AT stimulation with release of histamine [80]. In macrophages, neutrophils, and eosinophils, C3a and C5a can trigger oxidative burst [81, 84].

Newly generated over hours

Newly generated over hours mediators are mainly growth factors, cytokines, and chemokines. Specific mediators released by MC may have autocrine, paracrine, and endocrine effects of interest to the pathogenesis of anaphylaxis. The cytokines IL-8 and GM-CSF have paracrine effects on other cells of leukocyte lineage, including eosinophils, neutrophils, and macrophages [83]. Similarly, the expression of stem cell factor by MC can have facilitatory autocrine effects on mast cell survival, chemotaxis, growth, and proliferation [84, 85]. Another paracrine loop is mast cell-derived histamine binding to endothelial H1-receptors and leading to the elaboration of NO which contributes to vasodilation [86].

Stone et al. [87] analyzed 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 tumor necrosis factor (TNF)-alpha and TNFRI] and chemokines in a prospective study in patients who suffered anaphylaxis. All these mediators were higher in anaphylactic patients compared to control group. In this study, only tryptase, histamine, IL-2, IL-6, IL-10, and TNFRI correlated with hypotension, a finding similar to that found by Brown et al. [88]. Moreover, IL-6 and other inflammatory cytokines such as IL-8, TNF-a,

interferon gamma (IFN-g), and IL-1b induce the inactivation of cadherin, which mediate cell adhesion, leading to vascular leakage by increased capillary permeability. It has been postulated that IL-1 released from monocytes and macrophages activates endothelial expression of NO leading to this capillary permeability. On the other hand, recently, the role of IL-6 has been described as an excellent biomarker of cytokine release reactions because of its correlation with the severity of the reactions and its longevity in a blood serum. In addition, it has been demonstrated the role of IL-6 in the systemic inflammatory response syndrome (SIRS) [89]. There are promising data regarding major basophil chemotactic factor, chemokine (C-C motif) ligand 2, or CCL-2. Concentration of CCL-2 has been found to be significantly higher in anaphylaxis compared to healthy control cohort, and potentially identified severe anaphylaxis cases [65]. Latest studies, including human and murine models, have detailed several novel mediators that may participate in anaphylaxis reaction [30, 83, 90], as it has been summarized in Table 2.

Conclusions

Pathophysiology of anaphylaxis involves the activation of multiple pathways beyond IgE, different cell types, and a wide range of mediators. Therefore, measuring only one mediator may not be enough to diagnose anaphylaxis. This could partly explain the current lack of optimal biomarkers to confirm the diagnosis.

Given that anaphylaxis remains a clinical diagnosis, there is still exist a challenge in the laboratory diagnosis of anaphylaxis. Biomarkers are useful to reinforce the diagnosis and distinguish anaphylaxis from similar clinical scenarios. New biomarkers are pointing their interest such as chymase, carboxypeptidaseA3, PAF, PAF-AH, IL-2, IL-6, IL-10, and CCL-2, providing sample requirements, assay platform, process, and costs. However, a consensus on a reference range about sample handling and processing requirements for different biomarkers is needed.

Further studies applying these approaches are needed and they might provide greater insight into factors determining severity, clinical risk stratification, identification of mast cell disorders, and improving our understanding of this relatively complex acute condition.

Declarations

Conflict of Interest

The authors declare no competing interests.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human participants or animal performed by any of the authors.

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