

# Mediators Released During Human Anaphylaxis

Shelley F. Stone · Simon G. A. Brown

© Springer Science+Business Media, LLC 2011

**Abstract** A range of mediators are generated during anaphylaxis, with redundancy of effects, multiple overlapping pathways, and involvement of several cell types. Key steps in the reaction occur at the site of initial contact, and mediators may not be detectable systemically. Furthermore, the potencies of various mediators vary enormously, and clinical effects may occur below our level of detection. We also do not know what converts (amplifies) a local reaction into systemic anaphylaxis. Murine models have identified several novel mediators that may propagate and/or regulate this process and also indicate that circulating neutrophils may play an important role in reaction amplification. Differential expression of various genes within specific intracellular signalling pathways of mediator release may further explain the varying severities of anaphylactic reactions. As our knowledge of the mechanisms of activation, key mediators, and the regulation of mediator release improves, new treatments for prevention and acute management may emerge.

**Keywords** Anaphylaxis · Mast cells · Basophils · Mast cell tryptase · Histamine · Cytokines · Chemokines · Interleukin · Platelet-activating factor · IgE · IgG · Anaphylatoxins · Chymase · Prostaglandin · Leukotrienes · Regulation of signalling · FcεRI

---

S. F. Stone (✉) · S. G. A. Brown  
Centre for Clinical Research in Emergency Medicine,  
Western Australian Institute for Medical Research,  
University of Western Australia,  
Level 5 MRF Building, Rear 50 Murray Street,  
Perth, Western Australia 6000, Australia  
e-mail: Shelley.Stone@uwa.edu.au

S. G. A. Brown  
e-mail: Simon.Brown@uwa.edu.au

S. F. Stone · S. G. A. Brown  
Department of Emergency Medicine Royal Perth Hospital,  
GPO Box X2213, Perth, Western Australia 6001, Australia

## Introduction

Anaphylaxis is an immediate-type generalized hypersensitivity reaction affecting multiple organ systems, characterized at its most severe by bronchospasm, hypoxemia, and hypotension. It is defined simply as a “serious allergic reaction that is rapid in onset and may cause death” [1, 2].

More than 100 years after Portier and Richet first described it, anaphylaxis remains “under-recognized, under-reported, under-treated and poorly understood” [3]. We simply do not understand 1) what causes a localized allergic response to become rapidly generalized; 2) why only some individuals with high levels of allergen-specific antibodies experience anaphylaxis after allergen exposure; and 3) why a patient with clinical reactivity can present with markedly varying reaction severities after subsequent allergen exposures, ranging from a mild skin reaction alone to life-threatening anaphylaxis [3]. We also do not understand how immunotherapy works so well to prevent anaphylaxis for some allergens.

The incidence of anaphylaxis appears to have increased in the past 10 to 15 years, specifically food-related anaphylaxis in children and adolescents and anaphylaxis to drugs in adults [4–8]. The effects of allergy and anaphylaxis on quality of life [9–15] and health system expenditure are significant [16–19]. Peanut allergy remains the leading cause of death in food-allergic individuals [20, 21] and drug-related anaphylaxis deaths increased 300% in Australia from 1997 to 2005 [8]. Fatalities are often attributed to delays in adrenaline administration, but even patients who receive prompt treatment do not always survive. Therefore, of arguably greater importance is the issue of primary prevention (~50% of anaphylaxis deaths occur with the first reaction) and secondary prevention of recurrent episodes. Improving our understanding of the mechanisms of acute anaphylaxis, with specific focus on

the regulation of the amplification of the allergic signal and subsequent mediator production, is paramount for designing strategies to reduce the risk of reactions and has been identified as a key research priority [2].

This review discusses the production of anaphylactic mediators during anaphylaxis, the involvement of effector and target cells in mediator production, and the regulation of mediator production by specific cell signalling pathways. The clinical usefulness of measuring particular mediators in human anaphylaxis is also addressed. This review summarizes important publications from the past 12 months and discusses concepts of how the regulation and production of particular mediators may influence the severity and clinical symptoms of human anaphylaxis, and how this knowledge might be applied in future research.

## Triggers

### IgE-Mediated Allergic Anaphylaxis

Sensitization to an allergen occurs when naive CD4<sup>+</sup> Th0 cells differentiate into Th2 cells and secrete cytokines such as interleukin (IL)-4, IL-5, and IL-13 that stimulate B cells to produce allergen-specific IgE (sIgE). Allergen-specific IgE binds to its high-affinity receptor, FcεRI, expressed on effector cells, predominantly mast cells and basophils [22]. In humans, FcεRI complexes are expressed on several additional cell types, including monocytes/macrophages, Langerhans cells, myeloid and plasmacytoid dendritic cells (DCs), eosinophils, neutrophils, platelets, and bronchial smooth muscle cells.

In humans, anaphylaxis is predominantly triggered when effector cells sensitized with sIgE are re-exposed to allergen. Binding of minute quantities of allergen cross-links FcεRI, causing the release of preformed and newly synthesized biochemical mediators, including mast cell tryptase (MCT), histamine, prostaglandins, leukotrienes, and numerous cytokines and chemokines [23]. Individuals with high levels of sIgE and therefore high levels of FcεRI expression on the surface of mast cells and basophils may have effector cells primed to be more sensitive to allergen and to release larger amounts of mediators in response to allergen. However, sensitized individuals with high levels of sIgE may exhibit no clinical reactivity, and patients with undetectable or very low sIgE and barely positive skin tests can experience life-threatening reactions. IgE specificity may be important, and other poorly understood factors probably play a role in determining when sIgE sensitization translates into clinical reactivity. Another complicating factor relevant to drug allergy is that sIgE (and IgG) may be directed at drug metabolites rather than the drug itself, although this mechanism remains speculative [24].

### Non-IgE-Mediated Anaphylaxis

Several other immunologic (allergic) and nonimmunologic (nonallergic) mechanisms of anaphylaxis have also been proposed [25–27]. These include complement generation (anaphylatoxins C3a and C5a), coagulation system activation, neuropeptide release, immune complex formation, cytotoxicity, and leukocyte activation (T cells, DCs, and monocytes). For example, the ability of some substances to rapidly activate complement, with production of large amounts of the anaphylatoxin C3a, may stimulate macrophages, basophils, and mast cells to secrete platelet-activating factor (PAF) and histamine, which contribute to increased vascular permeability [28]. In addition, PAF can induce histamine release from mast cells in the absence of allergen by binding to the PAF receptor [29]. It has been difficult to confirm the contribution of allergen-specific IgG to human anaphylaxis, but it has been proposed that IgG-mediated anaphylaxis in humans requires exposure to very large doses of antigen, such as reactions to infused drugs such as antivenoms and contrast media. Khodoun et al. [30•] compared various blood markers between IgE- and IgG-mediated anaphylaxis in mice to potentially determine whether IgG-mediated anaphylaxis occurs in humans. Their observations suggested that decreased blood neutrophil FcγRIII expression without increased IL-4Rα expression may help facilitate determination of whether IgG immune complexes are responsible [30•].

About one in six adverse drug reactions represent drug hypersensitivity, which can be allergic or nonallergic [31]. Interestingly, up to 50% of patients with drug-induced anaphylaxis claim no prior contact with the triggering drug. This may suggest a non-IgE-mediated reaction but may also indicate prior unrecognized exposure or cross-reactivity between drug compounds and other allergens. Chemical compounds bound in a drug–protein complex may be recognized by the innate immune system (DCs and other cells) as a danger signal, resulting in upregulation of co-stimulatory molecules and cytokine production. These complexes may also generate new antigenic determinants, which are recognized by the adaptive immune system (T and B cells). Drug-specific IgG may lead to cell destruction (possibly by complement-mediated lysis) and sequestration. Immune complexes and anaphylatoxins generated by complement activation may lead to recruitment and activation of inflammatory cells, particularly neutrophils. Direct activation of mast cells by drugs (eg, morphine and codeine) may also occur.

### Pathophysiology

Anaphylaxis is manifest clinically by erythema (flushing), angioedema (swelling), bronchospasm and airway edema (wheeze), hypoxemia (cyanosis), cramping abdominal pain,

and collapse (unconsciousness) due to arterial hypotension. Arterial hypotension (shock) probably results from a mixture of reduced venous return to the heart (due to venodilation and hypovolemia from fluid extravasation), altered cardiac function (due to reflex reduction in heart rate and reduced myocardial contractility), and reduced after-load (due to arterial dilation) [32]. All these changes result primarily from the effect of biochemical mediators that cause 1) blood vessel dilation, 2) extravasation of fluid from the circulation into tissues, 3) smooth muscle constriction, and 4) myocardial dysfunction.

## Mediators

Mediators of anaphylaxis traditionally have been placed in three classes: 1) preformed mediators stored in the cells'

cytoplasmic granules released by degranulation (ie, histamine, proteases, proteoglycans, and some cytokines and growth factors); 2) newly synthesized proinflammatory lipid mediators (ie, prostaglandins and leukotrienes); and 3) newly synthesized growth factors, cytokines, and chemokines. The known physiologic effects of mediators associated with human anaphylaxis are summarized in Table 1. More recently, murine models have identified several novel mediators that may propagate and/or regulate the immune response. These include sphingosine-1-phosphate, galectin-9, IL-33, and nitric oxide [23, 33–37].

Analyzing the roles of these mediators in human disease is difficult because of interspecies variation, the opportunistic nature of anaphylaxis research, and the ethical issues surrounding deliberate provocation of anaphylaxis. Interpretation of the available data is also hampered by the fact that key steps in the allergic reaction occur locally at the

**Table 1** Mediators of anaphylaxis

| Mediator                                                                                         | Known or possible effects                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Preformed for immediate release                                                                  |                                                                                                                                                                                                                                                                                            |
| Histamine                                                                                        | Vasodilation and edema, bronchoconstriction, mucus secretion, nerve stimulation, reduced myocardial contractility (H1 receptor), increased myocardial contractility (H2 receptor)                                                                                                          |
| Heparin                                                                                          | Anticoagulant, anti-inflammatory, mediates capillary leakage and edema formation by initiating the formation of bradykinin (a vasoactive and proinflammatory peptide hormone)                                                                                                              |
| Tryptase                                                                                         | Amplification of allergic response (positive feedback on effector cells), leukocyte migration and activation, bronchoconstriction, vasodilation and edema, tissue degradation, and cell proliferation ("remodeling")                                                                       |
| Chymase                                                                                          | Vasodilation and edema, mucus secretion, leukocyte activation, tissue degradation                                                                                                                                                                                                          |
| TNF- $\alpha$                                                                                    | Bronchoconstriction, leukocyte adhesion, leukocyte migration and activation, possible role in delayed-phase reactions                                                                                                                                                                      |
| Newly generated over minutes                                                                     |                                                                                                                                                                                                                                                                                            |
| Cyclooxygenase products, mainly PGD <sub>2</sub>                                                 | Vasodilation and edema, bronchoconstriction, mucus secretion, nerve stimulation ( $\rightarrow$ vasodilation, itching, bronchoconstriction)                                                                                                                                                |
| Lipoxygenase products: LTB <sub>4</sub> , LTC <sub>4</sub> , LTD <sub>4</sub> , LTE <sub>4</sub> | Vasodilation and edema, mucus secretion, bronchoconstriction, leukocyte recruitment                                                                                                                                                                                                        |
| PAF                                                                                              | Platelet activation/microthrombi, leukocyte migration/activation, histamine release (indirectly by neurogenic activation), reduced myocardial contractility                                                                                                                                |
| Newly generated over hours                                                                       |                                                                                                                                                                                                                                                                                            |
| IL-5, GM-CSF                                                                                     | Leukocyte adhesion, leukocyte migration and activation                                                                                                                                                                                                                                     |
| IL-4, IL-13                                                                                      | IgE production and upregulation of Fc $\epsilon$ RI expression                                                                                                                                                                                                                             |
| IL-10                                                                                            | Anti-inflammatory, reduces activation and degranulation of mast cells, induces increased numbers of Tregs                                                                                                                                                                                  |
| IL-6                                                                                             | Proinflammatory cytokine; correlates with the extent of erythema and inversely related to mean arterial pressure; correlates strongly with occurrence of hypotension; causes increased expression of Fc $\epsilon$ RI, increased intracellular histamine, and prevents mast cell apoptosis |
| sTNFRI                                                                                           | Surrogate marker for TNF- $\alpha$ activity, may have anti-inflammatory effects                                                                                                                                                                                                            |
| PAF-AH                                                                                           | Enzyme that inactivates PAF, low levels reported in fatal anaphylaxis                                                                                                                                                                                                                      |
| Anaphylatoxins (C3a, C4a, C5a)                                                                   | Complement activation products, mast cell and neutrophil degranulation, and smooth muscle contraction                                                                                                                                                                                      |
| Chemokines (ie, RANTES, IL-8, MCP-1)                                                             | Chemotaxis and activation of immune cells, histamine and serotonin release from mast cells                                                                                                                                                                                                 |

*GM-CSF* granulocyte-macrophage colony-stimulating factor; *IL* interleukin; *LT* leukotriene; *MCP-1* monocyte chemoattractant protein-1; *PAF* platelet-activating factor; *PAF-AH* PAF acetylhydrolase; *PG* prostaglandin; *RANTES* regulated on activation, normal T-cell expressed and secreted; *sTNFRI* soluble tumor necrosis factor receptor I; *TNF* tumor necrosis factor; *Treg* regulatory T cell

site of initial contact and mediators from this component will not be detectable systemically. Furthermore, the relative potencies of various mediators vary enormously. For example, sulfidoleukotrienes are about 1,000 times more potent than histamine with regard to airway and vascular effects [38, 39]. Therefore, significant clinical effects may occur below the threshold of detection in blood, depending on the analytical technique used. Variation in target organ sensitivities and levels of regulatory mediators and hormones between individuals may also complicate the analysis of mediator levels.

A degree of redundancy in mediator effects is evident; this redundancy, multiple overlapping pathways, and probable involvement of several cell types has led to the concept of a mast cell–leukocyte cytokine cascade that initiates, amplifies, and perpetuates the allergic response [40]. The clinical relevance is that blockade of any single mediator alone is unlikely to have any significant therapeutic effect; thus, we use physiologic antagonism with epinephrine, an effective cardiac stimulant and bronchodilator. Epinephrine also reduces fluid extravasation from capillaries and in higher doses will constrict blood vessels.

Increased blood levels of several mediators have been associated with reaction severity in humans, including PAF, IL-6, IL-10, soluble tumor necrosis factor (TNF) receptor I, MCT, and histamine [41, 42]. Vadas et al. [42] also demonstrated low levels of PAF acetylhydrolase, which is responsible for degradation of PAF, in patients with fatal peanut anaphylaxis.

### Cellular Sources of Mediators of Anaphylaxis

Tissue mast cells and circulating basophils are thought to represent the major source of mediators that contribute to the pathology of anaphylaxis [43]. However, it is difficult to define the exact roles of each potential effector cell (also including monocytes/macrophages, DCs, neutrophils, and eosinophils) in IgE- and non-IgE-mediated anaphylaxis in humans.

#### Mast Cells

Human mast cells are generally considered to be the primary effector cells of anaphylaxis, producing all the mediators described in Table 1. Mast cells are found in the skin, lungs, gastrointestinal tract, and heart. Expression of particular mast cell mediators differs among mast cells in different tissue locations, and expression differs again between murine models and humans [44]. For example, the histamine content of human heart mast cells is comparable to that of lung and skin mast cells, whereas the tryptase content of human heart mast cells is lower than

that of skin mast cells and higher than that of lung mast cells [45•]. The effects of some mediators, such as C5a, may be restricted due to differential expression of their receptors on different populations of mast cells. Therefore, the severity and symptoms of anaphylaxis may be partially dependent on the route of allergen exposure (ie, ingested or injected) and the tissue mast cell population activated.

#### Role of Other Immune Cells and Target Cells

The mechanisms behind the rapid systemic spread of immune activation and mediator release in human anaphylaxis are not well-understood, with our current knowledge of mast cell signalling identified primarily through experiments using mast cells from genetically modified mice [46]. While mast cells have long been the focus of anaphylaxis research, it is becoming increasingly clear that the spectrum of immune cells and mediators involved in acute anaphylactic reactions is more complex than originally proposed. Mast cell–deficient and FcεRI knockout mice are able to mount anaphylactic responses to allergens [25, 47, 48]. Although basophils and DCs are minor populations in peripheral blood, their role in propagating the systemic manifestations of anaphylaxis cannot be discounted. Both populations express FcεRI and anaphylatoxin receptors and are capable of releasing large amounts of potent proinflammatory mediators, including PAF, TNF-α, and IL-6, into the circulation [49, 50]. Basophils also contribute to anaphylaxis by releasing histamine and leukotriene C4 [26]. Basophils have been shown to be dispensable for IgE-mediated anaphylaxis but play a crucial role in IgG-mediated anaphylaxis in a murine model through their release of PAF (instead of histamine) 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 the severity of an allergic reaction [51]. Basophil activation tests, measuring the *in vitro* degranulation of basophils through mediator release or changes in activation markers (CD63, CD203c) by flow cytometry after allergen-specific stimulation, have been promoted as tools for diagnosing allergy and monitoring the effects of immunotherapy [49]. Their ability to predict patients at risk of severe reactions is still to be confirmed, and the regulatory processes that determine the degree of basophil responsiveness during an acute reaction are not known.

T cells and monocytes may also play a role in the propagation or regulation of systemic anaphylaxis. Allergen-specific T cells are present in the circulation of allergic individuals, but their role during acute anaphylactic reactions is not known. In humans, both the absolute number and percentage of activated T cells can increase during anaphylaxis [52, 53]. Peanut-specific T cells

proliferate after peanut-induced anaphylaxis in peanut-allergic mice [54]. Activation and mediator release by T cells and monocytes may be important during reactions to particular allergens through direct interactions between immune receptors and small molecules bound to carrier proteins [31]. The induction of regulatory T cells (Tregs) plays an important role in successful venom immunotherapy, suggesting these cells may regulate allergic reactions in sensitized individuals [55]. Elevated serum levels of the regulatory cytokine IL-10 in patients with severe anaphylaxis suggests a possible role for Tregs in anaphylaxis, although mast cells are also a potential source of IL-10 [41].

Macrophages have been reported to play a major role in IgG-mediated systemic anaphylaxis through the release of PAF. Selective deletion of mast cells and macrophages, but not mast cells and basophils, averted nearly all clinical and physiologic signs of peanut-induced anaphylaxis in a murine model [56•].

The role of DCs in anaphylaxis remains largely unknown. In 2010, Zhou et al. [57] published a study demonstrating that the induction of oral tolerance in a prophylactic model of food-induced anaphylaxis was enhanced through signalling through the C-type lectin receptor SIGNR-1 expressed on DCs in the gastrointestinal lamina propria. They hypothesized that disruption of gastrointestinal homeostasis due to defective mucosal DC responses and reduced Treg production may result in defective tolerance and the subsequent symptoms of anaphylaxis upon exposure to particular allergens.

Neutrophils are the most abundant cells among blood leukocytes in humans. Jonsson et al. [58••] demonstrated that neutrophils are activated during particular murine models of anaphylaxis, that neutrophil depletion inhibited active and passive anaphylaxis, and that administration of both murine and human neutrophils was then able to restore anaphylactic potential. Neutrophils secrete several mediators that may contribute to anaphylaxis, including PAF and histamine.

Endothelial cells and smooth muscle cells are target cells for mediators released by immune cells during anaphylaxis. However, the contribution of mediators released by these cells—downstream of immune cell activation—to the severity and symptoms of anaphylaxis has not been investigated extensively. Endothelins are a family of peptides produced by endothelial cells in response to injury. Mature endothelins act by engaging receptors on the surface of a variety of cells, including vascular smooth muscle and mast cells, which can contribute to vasoconstriction, mast cell activation, and vascular remodelling. Human heart mast cells activated by anti-IgE release a substance that converts big endothelin to endothelin-1 (ET-1), a potent bronchoconstrictor [45••]. Human chymase processes precursor “big” endothelins into a novel, bron-

choconstricting form, ET-1(1–31) [59]. Human mast cells may be involved with both activating and inactivating endothelin, depending on the activating stimulus (ie, allergen). Mast cells express the endothelin-A receptor, and binding of ET-1 induces rapid degranulation [60]. Therefore, a positive feedback loop between activation of mast cells and endothelial cells may increase the severity of anaphylaxis.

### Regulation of FcεRI Signalling, Mast Cell Function, and Mediator Release

Intracellular signalling pathways that regulate—both positively and negatively—mediator release from mast cells and other effector and end-organ cells may be critical for understanding the mechanisms and amplification of allergic reactions. Early activation events during FcεRI-triggered mediator release after mast cell activation include the production of reactive oxygen species, Ca<sup>2+</sup> mobilization, and phosphorylation of signalling molecules. Later events include changes in expression of potentially hundreds of genes, including cytokines and chemokines. For example, a microarray study of cultured human mast cells 2 h after FcεRI cross-linking found that the level of expression of 1,771 genes increased by at least 10% [61••]. In the past 12 months, several published studies have continued to provide new data regarding regulation of FcεRI signalling and mediator release during anaphylaxis.

In mast cells, phosphoinositol-3 (PI3) kinase has been shown to regulate cell proliferation, adhesion, and migration, as well as antigen-IgE-induced degranulation and cytokine release [62]. PI3 kinase activates the serum- and glucocorticoid-inducible kinase 3 (SGK-3), which plays a role in the stimulation of mast cell degranulation. Zemtsova et al. [63] demonstrated that mast cell secretion of histamine and β-hexosaminidase is impaired in gene-targeted mice lacking SGK-3.

Samayawardhena and Pallen [64] investigated the role of receptor protein tyrosine phosphatase (PTP)α, an activator of Src family kinases Lyn, Fyn, and Hck, which are critical FcεRI-proximal regulators of signalling during mast cell activation. Using PTPα knockout mice, they demonstrated that PTPα activates the FcεRI-coupled kinases Lyn and Fyn and suppresses Hck activity, resulting in enhanced IgE-dependent allergic responses in PTPα<sup>−/−</sup> mice [64]. PTPα deficiency led to increased IL-2, IL-6, TNF-α, and cysteinyl leukotriene production by activated mast cells. Another Src family kinase, Fgr, has been shown to be critical for activation of mast cells and IgE-mediated anaphylaxis in mice, as Fgr positively regulates degranulation, production of eicosanoids, and cytokines [65]. In addition, the absence of a number of Tec family kinases

severely impairs FcεRI-dependent mast cell responses [66].

Differential expression of various genes within specific pathways of mediator release may explain the heterogeneous symptoms of mast cell activation and the varying severities of anaphylactic reactions. Nishida et al. [67•] demonstrated that the presence of Gab2 affects mast cell degranulation, but not cytokine production, and demonstrated different requirements for the development of IgE-mediated anaphylaxis versus contact hypersensitivity. Differential regulation of mast cell degranulation and cytokine production, specifically IL-6, with distinct stimuli, resulting in the release of specific mediator pools, has also been demonstrated by McCall-Culbreath et al. [68].

## Clinical Usefulness of Measuring Mediators

### Initial Diagnosis of Anaphylaxis

Currently, only two mediators of anaphylaxis are measured in clinical laboratories—total MCT and histamine—but these tests are not usually available on an emergency basis and, when taken at a single time point, are neither specific nor completely sensitive for diagnosing anaphylaxis. Early serial measurements of mediator changes over time are more accurate than single measurements [41, 69]. Previous reports have suggested tryptase levels are rarely elevated during severe food-induced anaphylaxis, but we have found that mast cell tryptase increases in patients with severe food-induced anaphylaxis [41]. This highlights the importance of collecting serial blood samples. Nevertheless, despite having a high specificity (~98%), the sensitivity of serial MCT measurement remains at only about 73% for cases of severe anaphylaxis [41, 69], and diagnostic and clinical utility have yet to be confirmed in a large study. Anecdotally, we have found that a positive result can be helpful when the diagnosis of anaphylaxis is uncertain (eg, hypotensive shock without diagnostic skin features), but a negative result cannot be used to exclude the diagnosis.

### Postmortem Determination of Diagnosis of Fatal Anaphylaxis

In cases of sudden death due to suspected but unwitnessed exposure to allergen, postmortem diagnosis of fatal anaphylaxis is difficult and requires careful investigation. Postmortem measurement of mediator levels or expression of mediator receptors in target organs may assist with diagnosis. Moderately elevated tryptase levels are common in postmortem sera due to nonanaphylactic deaths, but values above 45 µg/mL may support the diagnosis of fatal anaphylaxis [70]. However, fatal anaphylaxis can occur in

the apparent absence of allergen-specific IgE and release of histamine and tryptase [71].

In a recent case of suspected fatal anaphylaxis due to a honeybee sting, Toro et al. [72] were able to demonstrate C5R1 expression in dilated capillaries in the lungs, specifically intensive cytoplasmic staining detected in endothelial cells, suggesting an active role for complement in fatal anaphylaxis. Analysis of tissue samples rather than blood should be considered, as the timing of blood samples after death can influence mediator levels [70]. A study of postmortem blood and tissue samples from eight patients who died of anaphylaxis demonstrated higher IgE levels in blood collected from the patients' heart compared with controls who died from other causes [73]. Levels of the regulatory proteins SOCS1 and SOCS3 were also significantly higher in the kidney and liver of individuals who died from anaphylaxis. Whether these novel approaches may be applicable as routine investigative tools remains unclear.

### Development of New Treatments

Our knowledge of the mechanisms of mast cell activation, the mediators influencing severity, and their regulation will aid the development and assessment of new treatments for anaphylaxis. Development of safe and effective treatments for food allergy is particularly important due to the poor success rate of trialled immunotherapies. Food Allergy Herbal Formula-2 (FAHF-2) has been found to block peanut-induced anaphylaxis in a murine model. Song et al. [74] demonstrated that this protection was due to a FAHF-2–induced reduction in basophil and mast cell numbers and suppression of IgE-mediated mast cell activation, including reduced FcεRI expression (particularly the γ subunit) and release of histamine after allergen challenge. Interestingly, increased interferon-γ production by CD8<sup>+</sup> T cells may be an important mechanism underlying FAHF-2–induced protection. In a recent acute phase 1 study of patients with food allergy, 6 months of thrice-daily doses of FAHF-2 was shown to be safe and well-tolerated and resulted in a significant reduction in basophil CD63 expression in response to ex vivo stimulation [75].

Using the rapidly evolving field of nanomedicine, Norton et al. [61••] tested a panel of fullerene derivatives for their ability to prevent the release of allergic mediators. They reported a new compound that significantly inhibited degranulation and cytokine production from mast cells and basophils and was able to block mast cell–driven anaphylaxis in vivo. They also demonstrated that the early phase of FcεRI inhibition was dependent on the blunted release of intracellular calcium stores, elevations in reactive oxygen species, and in several signalling molecules.

Several new potential inhibitors of mast cell mediator release have been reported in the past 12 months, including endogenous compounds and cell surface receptors. These include the Ras inhibitor farnesylthiosalicylic acid, which reduces prostaglandin D<sub>2</sub> and TNF- $\alpha$  production [76]; chrysin, which decreases histamine, TNF- $\alpha$ , IL-1 $\beta$ , IL-4, and IL-6 production [77]; allergin-1, an immunoglobulin-like receptor expressed on mast cells [78]; and U63A05, a novel synthetic compound that inhibits Syk activation in mast cells and suppresses degranulation and TNF- $\alpha$  and IL-4 production [79].

## Conclusions

In the early 20th century, histamine was thought to be the principal mediator of anaphylaxis. Since then, an ever-expanding range of mediators have been implicated by human studies, in vitro cell stimulation studies, and animal models. It is now clear that anaphylaxis involves the action of multiple immune effector pathways and cell types, and intervention targeting a single pathway may not be sufficient to prevent anaphylactic responses. Identification of key pathways that contribute to the amplification of the allergic signal early during anaphylaxis and primary interventions to prevent these catastrophic reactions are a research priority.

**Disclosure** No potential conflicts of interest relevant to this article were reported.

## References

1. Sampson HA, Munoz-Furlong A, Campbell RL, et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *Ann Emerg Med*. 2006;47(4):373–80.
2. Sampson HA, Munoz-Furlong A, Campbell RL, et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol*. 2006;117(2):391–7.
3. Golden DB. What is anaphylaxis? *Curr Opin Allergy Clin Immunol*. 2007;7(4):331–6.
4. Poulos LM, Waters AM, Correll PK, et al. Trends in hospitalizations for anaphylaxis, angioedema, and urticaria in Australia, 1993–1994 to 2004–2005. *J Allergy Clin Immunol*. 2007;120(4):878–84.
5. Rudders SA, Banerji A, Vassallo MF, et al. Trends in pediatric emergency department visits for food-induced anaphylaxis. *J Allergy Clin Immunol*. Aug;126(2):385–8.
6. Mullins RJ, Dear KB, Tang ML. Characteristics of childhood peanut allergy in the Australian Capital Territory, 1995 to 2007. *J Allergy Clin Immunol*. 2009;123(3):689–93.
7. Mullins RJ. Paediatric food allergy trends in a community-based specialist allergy practice, 1995–2006. *Med J Aust*. 2007;186(12):618–21.
8. Liew WK, Williamson E, Tang ML. Anaphylaxis fatalities and admissions in Australia. *J Allergy Clin Immunol*. 2009;123(2):434–42.
9. Resnick ES, Pieretti MM, Maloney J, et al. Development of a questionnaire to measure quality of life in adolescents with food allergy: the FAQL-teen. *Ann Allergy Asthma Immunol*. 2010;105(5):364–8.
10. Sampson MA, Munoz-Furlong A, Sicherer SH. Risk-taking and coping strategies of adolescents and young adults with food allergy. *J Allergy Clin Immunol*. 2006;117(6):1440–5.
11. Mullins RJ. Anaphylaxis: risk factors for recurrence. *Clin Exp Allergy*. 2003;33(8):1033–40.
12. Marklund B, Ahlstedt S, Nordstrom G. Food hypersensitivity and quality of life. *Curr Opin Allergy Clin Immunol*. 2007;7(3):279–87.
13. Bollinger ME, Dahlquist LM, Mudd K, et al. The impact of food allergy on the daily activities of children and their families. *Ann Allergy Asthma Immunol*. 2006;96(3):415–21.
14. Hu W, Kerridge I, Kemp A. Risk, rationality, and regret: responding to the uncertainty of childhood food anaphylaxis. *Med Humanit*. 2005;31(1):12–6.
15. Oude Elberink JN, Dubois AE. Quality of life in insect venom allergic patients. *Curr Opin Allergy Clin Immunol*. 2003;3(4):287–93.
16. Simons FE. Epinephrine auto-injectors: first-aid treatment still out of reach for many at risk of anaphylaxis in the community. *Ann Allergy Asthma Immunol*. 2009;102(5):403–9.
17. Mullins RJ, Clark S, Camargo Jr CA. Regional variation in epinephrine autoinjector prescriptions in Australia: more evidence for the vitamin D-anaphylaxis hypothesis. *Ann Allergy Asthma Immunol*. 2009;103(6):488–95.
18. Mills EN, Mackie AR, Burney P, et al. The prevalence, cost and basis of food allergy across Europe. *Allergy*. 2007;62(7):717–22.
19. Access Economics. The economic impact of allergic disease in Australia: not to be sneezed at. 2007 13th of November:1–111.
20. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001–2006. *J Allergy Clin Immunol*. 2007;119(4):1016–8.
21. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol*. 2001;107(1):191–3.
22. Peavy RD, Metcalfe DD. Understanding the mechanisms of anaphylaxis. *Curr Opin Allergy Clin Immunol*. 2008;8(4):310–5.
23. Ben-Shoshan M, Clarke AE. Anaphylaxis: past, present and future. *Allergy*. 2011;66(1):1–14.
24. Harrer A, Lang R, Grims R, et al. Diclofenac hypersensitivity: antibody responses to the parent drug and relevant metabolites. *PLoS One*. 2010;5(10):e13707.
25. Finkelman FD. Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol*. 2007;120(3):506–15.
26. Karasuyama H, Tsujimura Y, Obata K, Mukai K. Role for basophils in systemic anaphylaxis. *Chem Immunol Allergy*. 2010;95:85–97.
27. Simons FE. Anaphylaxis pathogenesis and treatment. *Allergy*. 2011;66 Suppl 95:31–4.
28. Khodoun M, Strait R, Orekov T, et al. Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol*. 2009;123(2):342–51.
29. Kajiwarana N, Sasaki T, Bradding P, et al. Activation of human mast cells through the platelet-activating factor receptor. *J Allergy Clin Immunol*. May;125(5):1137–45.

30. • Khodoun MV, Strait R, Armstrong L, et al. Identification of markers that distinguish IgE- from IgG-mediated anaphylaxis. *Proc Natl Acad Sci U S A*. 2011;108(30):12413–8. *Decreased blood neutrophil FcγRIII expression without IL-4Rα expression may be used to determine whether and when IgG-mediated anaphylaxis occurs in humans.*
31. Pichler WJ, Adam J, Daubner B, et al. Drug hypersensitivity reactions: pathomechanism and clinical symptoms. *Med Clin North Am*. 2010;94(4):645–64.
32. Brown SG. The pathophysiology of shock in anaphylaxis. *Immunol Allergy Clin North Am*. 2007;27(2):165–75.
33. Oskeritzian CA, Price MM, Hait NC, et al. Essential roles of sphingosine-1-phosphate receptor 2 in human mast cell activation, anaphylaxis, and pulmonary edema. *J Exp Med*. 2010;207(3):465–74.
34. Niki T, Tsutsui S, Hirose S, et al. Galectin-9 is a high affinity IgE-binding lectin with anti-allergic effect by blocking IgE-antigen complex formation. *J Biol Chem*. 2009;284(47):32344–52.
35. Sade K, Schwartz IF, Etkin S, et al. Expression of inducible nitric oxide synthase in a mouse model of anaphylaxis. *J Investig Allergol Clin Immunol*. 2007;17(6):379–85.
36. Hsu CL, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PLoS One*. 2010;5(8):e11944.
37. Olivera A, Rivera J. An emerging role for the lipid mediator sphingosine-1-phosphate in mast cell effector function and allergic disease. *Adv Exp Med Biol*. 2011;716:123–42.
38. Handley DA, Farley C, Deacon RW, Saunders RN. Evidence for distinct systemic extravasation effects of platelet activating factor, leukotrienes B<sub>4</sub>, C<sub>4</sub>, D<sub>4</sub> and histamine in the guinea pig. *Prostaglandins Leukot Med*. 1986;21(3):269–77.
39. Christie PE, Schmitz-Schumann M, Spur BW, Lee TH. Airway responsiveness to leukotriene C<sub>4</sub> (LTC<sub>4</sub>), leukotriene E<sub>4</sub> (LTE<sub>4</sub>) and histamine in aspirin-sensitive asthmatic subjects. *Eur Respir J*. 1993;6(10):1468–73.
40. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol*. 2000;105(5):847–59.
41. Stone SF, Cotterell C, Isbister GK, et al. Elevated serum cytokines during human anaphylaxis: identification of potential mediators of acute allergic reactions. *J Allergy Clin Immunol*. 2009;124(4):786–92.
42. Vadas P, Gold M, Perelman B, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med*. 2008;358(1):28–35.
43. Kalesnikoff J, Galli SJ. Anaphylaxis: mechanisms of mast cell activation. *Chem Immunol Allergy*. 2010;95:45–66.
44. Caughey GH. Mast cell proteases as protective and inflammatory mediators. *Adv Exp Med Biol*. 2011;716:212–34.
45. •• Genovese A, Rossi FW, Spadaro G, et al. Human cardiac mast cells in anaphylaxis. *Chem Immunol Allergy*. 2010;95:98–109. *This is an excellent review of human heart mast cells in anaphylaxis, including discussion of differential mediator release and effects on endothelial cells and cardiovascular function.*
46. Metcalfe DD, Peavy RD, Gilfillan AM. Mechanisms of mast cell signaling in anaphylaxis. *J Allergy Clin Immunol*. 2009;124(4):639–46.
47. Okunuki H, Teshima R, Sakushima J, et al. Induction of active systemic anaphylaxis by oral sensitization with ovalbumin in mast-cell-deficient mice. *Immunol Lett*. 2000;74(3):233–7.
48. Sun J, Arias K, Alvarez D, et al. Impact of CD40 ligand, B cells, and mast cells in peanut-induced anaphylactic responses. *J Immunol*. 2007;179(10):6696–703.
49. Karasuyama H, Mukai K, Tsujimura Y, Obata K. Newly discovered roles for basophils: a neglected minority gains new respect. *Nat Rev Immunol*. 2009;9(1):9–13.
50. Soruri A, Kiafard Z, Dettmer C, et al. IL-4 down-regulates anaphylatoxin receptors in monocytes and dendritic cells and impairs anaphylatoxin-induced migration in vivo. *J Immunol*. 2003;170(6):3306–14.
51. MacGlashan Jr DW. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol*. 1993;91(2):605–15.
52. Grammer LC, Shaughnessy MA, Harris KE, Goolsby CL. Lymphocyte subsets and activation markers in patients with acute episodes of idiopathic anaphylaxis. *Ann Allergy Asthma Immunol*. 2000;85(5):368–71.
53. Yanagawa Y, Sakamoto T, Okada Y. Lymphocytosis without anemia in a patient presenting with anaphylactic shock. *Am J Emerg Med*. 2005;23(6):763–6.
54. Li XM, Serebrisky D, Lee SY, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol*. 2000;106(1 Pt 1):150–8.
55. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2007;119(4):780–91.
56. • Arias K, Chu DK, Flader K, et al. Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J Allergy Clin Immunol*. 2011;127(6):1552–61. *This study suggests pathways other than the classical mast cell–IgE pathway contribute to peanut-induced anaphylaxis, highlighting the importance of IgG and phagocytes.*
57. Zhou Y, Kawasaki H, Hsu SC, et al. Oral tolerance to food-induced systemic anaphylaxis mediated by the C-type lectin SIGNR1. *Nat Med*. 2010;16(10):1128–33.
58. •• Jonsson F, Mancardi DA, Kita Y, et al. Mouse and human neutrophils induce anaphylaxis. *J Clin Invest*. 2011;121(4):1484–96. *Neutrophil depletion inhibited active and passive anaphylaxis in mice and suggests neutrophil activation may contribute to anaphylaxis in humans. This requires confirmation in human anaphylaxis.*
59. Nakano A, Kishi F, Minami K, et al. Selective conversion of big endothelins to tracheal smooth muscle-constricting 31-amino acid-length endothelins by chymase from human mast cells. *J Immunol*. 1997;159(4):1987–92.
60. Metz M, Schafer B, Tsai M, et al. Evidence that the endothelin A receptor can enhance IgE-dependent anaphylaxis in mice. *J Allergy Clin Immunol*. 2011;128(2):424–6.
61. •• Norton SK, Dellinger A, Zhou Z, et al. A new class of human mast cell and peripheral blood basophil stabilizers that differentially control allergic mediator release. *Clin Transl Sci*. 2010;3(4):158–69. *This study identified a new class of compounds that interfere with mast cell and basophil activation and may provide a new approach for treating allergic disease. It also identified 1,771 genes with increased gene expression of more than 10% in mast cells after cross-linking of FcεRI.*
62. Ali K, Bilancio A, Thomas M, et al. Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. *Nature*. 2004;431(7011):1007–11.
63. Zemtsova IM, Heise N, Frohlich H, et al. Blunted IgE-mediated activation of mast cells in mice lacking the serum- and glucocorticoid-inducible kinase SGK3. *Am J Physiol Cell Physiol*. 2010;299(5):C1007–14.
64. Samayawardhena LA, Pallen CJ. PTPalpha activates Lyn and Fyn and suppresses Hck to negatively regulate FcεpsilonRI-dependent mast cell activation and allergic responses. *J Immunol*. 2010;185(10):5993–6002.
65. Lee JH, Kim JW, Kim do K, et al. The Src family kinase Fgr is critical for activation of mast cells and IgE-mediated anaphylaxis in mice. *J Immunol*. 2011;187(4):1807–15.
66. Iyer AS, Morales JL, Huang W, et al. Absence of Tec family kinases interleukin-2 inducible T cell kinase (Itk) and Bruton's

- tyrosine kinase (Btk) severely impairs Fc epsilonRI-dependent mast cell responses. *J Biol Chem.* 2011;286(11):9503–13.
67. • Nishida K, Yamasaki S, Hasegawa A, et al. Gab2, via PI-3K, regulates ARF1 in Fc{varepsilon}RI-mediated granule translocation and mast cell degranulation. *J Immunol.* 2011;187(2):932–41. *The presence of Gab2 affects mast cell degranulation, but not cytokine production, and the authors demonstrated different requirements for the development of IgE-mediated anaphylaxis versus contact hypersensitivity.*
  68. McCall-Culbreath KD, Li Z, Zhang Z, et al. Selective, alpha2-beta1 integrin-dependent secretion of IL-6 by connective tissue mast cells. *J Innate Immun.* 2011;3(5):459–70.
  69. Brown SG, Blackman KE, Heddle RJ. Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg Med Australas.* 2004;16(2):120–4.
  70. Mayer DE, Krauskopf A, Hemmer W, et al. Usefulness of post mortem determination of serum tryptase, histamine and diamine oxidase in the diagnosis of fatal anaphylaxis. *Forensic Sci Int.* 2011;212(1-3):96–101.
  71. Lin RY, Schwartz LB, Curry A, et al. Histamine and tryptase levels in patients with acute allergic reactions: an emergency department-based study. *J Allergy Clin Immunol.* 2000;106(1 Pt 1):65–71.
  72. Toro K, Borka K, Kardos M, et al. Expression and function of C5a receptor in a fatal anaphylaxis after honey bee sting. *J Forensic Sci.* 2011;56(2):526–8.
  73. Li Z, Liang Y, Tang H, et al. Effect of anaphylactic shock on suppressors of cytokine signaling. *Immunol Invest.* 2010;39(7):740–53.
  74. Song Y, Qu C, Srivastava K, et al. Food allergy herbal formula 2 protection against peanut anaphylactic reaction is via inhibition of mast cells and basophils. *J Allergy Clin Immunol.* 2010;126(6):1208–17.
  75. Patil SP, Wang J, Song Y, et al. Clinical safety of food allergy herbal formula-2 (FAHF-2) and inhibitory effect on basophils from patients with food allergy: extended phase I study. *J Allergy Clin Immunol.* 2011 (in press).
  76. Mor A, Ben-Moshe O, Mekori YA, Kloog Y. Inhibitory effect of farnesylthiosalicylic acid on mediators release by mast cells: preferential inhibition of prostaglandin D(2) and tumor necrosis factor-alpha release. *Inflammation.* 2011;34(5):314–8.
  77. Bae Y, Lee S, Kim SH. Chrysin suppresses mast cell-mediated allergic inflammation: involvement of calcium, caspase-1 and nuclear factor-kappaB. *Toxicol Appl Pharmacol.* 2011;254(1):56–64.
  78. Hitomi K, Tahara-Hanaoka S, Someya S, et al. An immunoglobulin-like receptor, allergin-1, inhibits immunoglobulin E-mediated immediate hypersensitivity reactions. *Nat Immunol.* 2010;11(7):601–7.
  79. Kim do K, Lee JH, Kim JW, et al. A novel imidazo[1,5-b]isoquinolinone derivative, U63A05, inhibits Syk activation in mast cells to suppress IgE-mediated anaphylaxis in mice. *J Pharmacol Sci.* 2011;115(4):500–8.