

Mediators of Anaphylaxis

Yoshiko Ogawa, MD*, J. Andrew Grant, MD,
FAAAAI, DFACAAI

*Division of Allergy, Pulmonary, Immunology, Critical Care, and Sleep [APICS],
University of Texas Medical Branch, Medical Research Building 8.104,
Galveston, TX 77555-1083, USA*

Anaphylaxis is a systemic, immediate hypersensitivity reaction that results from IgE-mediated release of vasoactive and inflammatory mediators from mast cells and basophils. The classic form, described in 1902 [1], involves prior sensitization to an allergen with later re-exposure, producing symptoms by means of an IgE-mediated immunologic mechanism. Clinical features in anaphylaxis are induced by the mediators released mainly by the high-affinity IgE receptor (FcεRI) cross-linking on mast cells and basophils previously sensitized by antigen (Fig. 1). Experimentally, mediators can be released by responding not only antigens, but also by anti-IgE, calcium ionophore A23187, compound 48/80, morphine sulfate, formyl-met-leu-phe (fMLP) peptide, substance P, anaphylatoxins, chemokines, complement fragments, and stimulation of toll-like receptors [2] (Fig. 2). The effects of IgE and FcεRI potentially can be reversed by IgG combining with the FcγRIIb receptor on basophils and mast cells [3]. Patients develop urticaria, angioedema, bronchospasm, hypotension, and gastrointestinal (GI) symptoms typically within an hour after mast cell activation.

In the early days, histamine was considered to be the principal mediator of anaphylaxis; however, it now is known that various mediators are involved. Preformed granule mediators are released by exocytosis within minutes. Synthesis of arachidonic acid (AA) metabolites including prostaglandins (PGs) and leukotrienes (LTs) also occurs within minutes, while activation of the synthesis of inflammatory cytokines and chemokines may take hours [2]. Typically, additional cells are recruited and activated during the late phase of allergic reactions, and these include basophils, eosinophils, and Th2 cells. A new approach to demonstrating basophil activation during

* Corresponding author.

E-mail address: yoogawa@utmb.edu (Y. Ogawa).

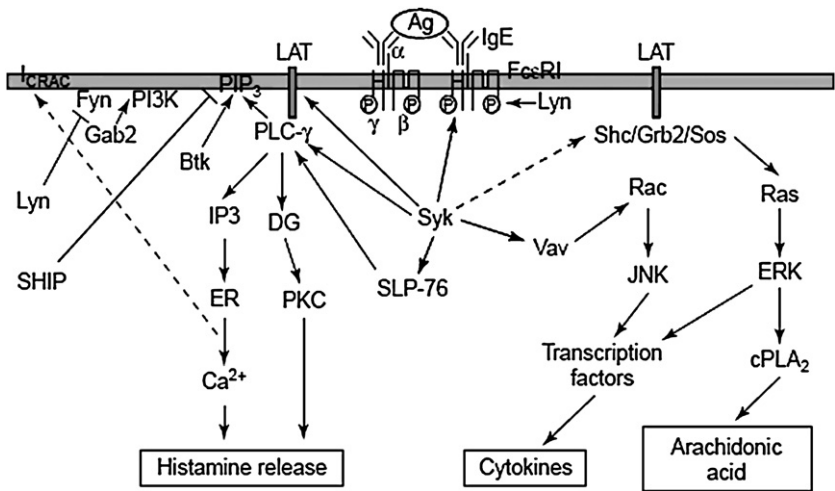


Fig. 1. FcεRI-mediated signaling pathways in mast cells. (Adapted from Siraganian RP. Mast cell signal transduction from the high-affinity IgE receptor. Curr Opin Immunol 2003;15:639-46; with permission.)

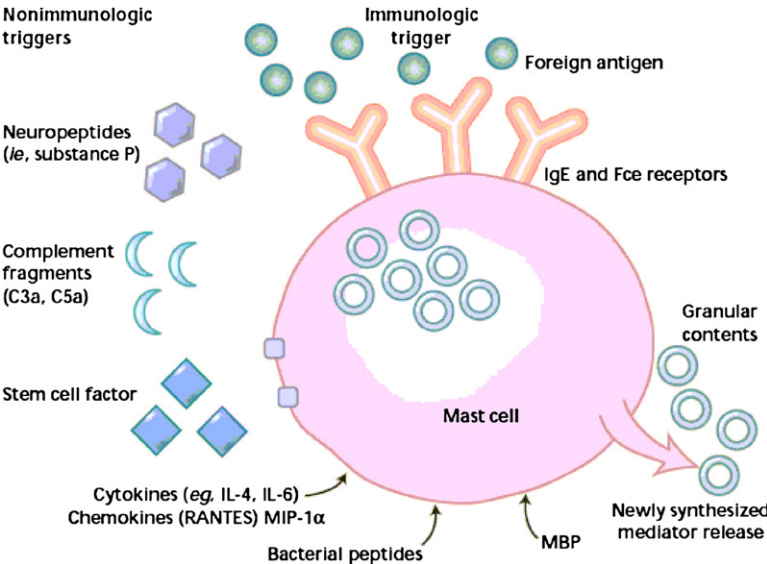


Fig. 2. Degranulation of mast cell. (Adapted from Grant JA, Hassan S, Leonard PA. Mast cell and basophil-derived mediators. Atlas of Allergic Diseases. 2nd edition. New York: Current Medicine Group; 2005. p. 1-10; with permission.)

anaphylactic reactions is use of flow cytometry and identification of surface markers of activation including CD63 and CD203c [4].

Approximately 84,000 cases of anaphylaxis occur in the United States every year, which affect 1.2 to 15% of the population. Among these cases, 500 to 1000 are fatal [5]. Therefore it is important to characterize the mediators of anaphylaxis in understanding the pathophysiology and treatment of anaphylaxis. This article reviews these mediators, first granule-associated preformed mediators, followed by membrane-derived lipid mediators, and then chemokines/cytokines.

Mediators from mast cells and basophils

Granule-associated preformed mediators

Histamine

Histamine is a critical mediator of the most rapidly occurring anaphylactic symptoms. Histamine is a diamine produced by decarboxylation of amino acid histidine in the Golgi apparatus of mast cell and basophils. Once in the extracellular environment, histamine is metabolized rapidly (half-life of 30 minutes) by histamine methyl transferase [6], which limits its use as a marker for mast cell and basophil activation. In an anaphylactic patient, plasma histamine begins to rise in 5 minutes and remains elevated only for 30 to 60 minutes. Urinary histamine metabolites, including methylhistamine, may be found for up to 24 hours after onset of anaphylaxis. The effects of histamine are mediated through H1, H2, H3, and H4 receptors, all of which are G protein-coupled receptors. This type of receptor is a heptahelical transmembrane molecule that can transduce extracellular signals by way of G proteins to intracellular second messenger systems. H1 receptors, followed by H2 receptors, are responsible for most anaphylactic reactions [7].

The physiologic phenomena that both H1 and H2 receptors exert with histamine stimulation are vasodilatation, increased vascular permeability, increased heart rate, increased cardiac contraction, and increased glandular secretion. Increased vascular permeability leads to urticaria and angioedema. Urticaria and angioedema are the most common clinical symptoms in anaphylaxis and are seen in 88% of cases [8]. Although platelet-activating factor (PAF) and arachidonic acid metabolites (prostaglandin D2, leukotrienes C and D) also contribute to urticaria and angioedema formation, histamine appears to be the major mediator.

Cardiac effects from histamine, such as enhanced inotropy and chronotropy, mainly are mediated by H2 receptors and also some effects from H1 receptors. H1 receptor stimulation increases the heart rate by shortening of sinoatrial node diastolic phase. H1 receptor stimulation also may cause coronary artery vasospasm and lead to myocardial infarction even in a patient who has normal coronary arteries [9].

When the H1 receptor is stimulated, it will lead to contraction of smooth muscles in the bronchial tree and GI tract. Wheezing and dyspnea are caused by H1 receptor stimulation and seen 55% to 60% of the time. GI symptoms such as nausea, vomiting, diarrhea, and cramping pain are seen in 25% to 30% of cases [10]. Increased mucous secretion is the result of increased mucous viscosity by H1 receptor stimulation and increased production by H2 receptor stimulation. It is seen as rhinorrhea and bronchorrhea.

H1 receptors on endothelial cells will stimulate the production of nitric oxide (NO), which indirectly will cause vasodilatation [10]. NO is produced from L-arginine by NO synthetase (NOS), and its actions are mediated by cyclic guanosine monophosphate (cGMP). There are three forms of the enzyme NOS; neuronal NOS (nNOS) is cloned from neurons, eNOS originally characterized in endothelial cells, and inducible NOS (iNOS), whose production appears to be inflammation-related and pathologic. Inducible NOS (iNOS) appears to be produced as an offshoot of the inflammatory response, by the stimulatory effects of tumor necrosis factor (TNF) and other cytokines. It results in massive production of NO, causing widespread vasodilatation (caused by loss of vasomotor tone) and hypotension. NO has these detrimental effects and beneficial effects, but studies are supporting the concept that NO inhibition will lead to better outcome in a shock state [11,12].

H2 receptors exert their effect directly on the vascular smooth muscle and cause vasodilatation. In anaphylactic shock, it has been shown that blockade of both H1 and H2 receptors is superior to H1 blockage alone [13,14]. Flushing, headache, increased pulse pressure, and increased diastolic pressure are controlled better with both H1 and H2 blockage.

There are recent studies showing that pruritus may be related the stimulation of the H3 receptors located in a brain [15]. Chrusch and colleagues [16] observed that a specific H3 receptor blocker thioperamide was effective in improving cardiac function in a dog model of anaphylaxis. H4 receptors, the newest member of the histamine receptor family, may play a role in inflammatory processes. A selective H4 receptor blocker was found to have anti-inflammatory effects in mice by blocking histamine-induced chemotaxis and calcium influx in mouse bone marrow-derived mast cells, and the histamine-induced migration of tracheal mast cells from the connective tissue toward the epithelium [17].

Neutral proteases

Tryptase

Tryptase is a serine esterase protein with a molecular weight of 110-130 kDa. Tryptase binds to heparin or other proteoglycans through its cationic groove. Heparin-stabilized tetramers of tryptase are stored mainly in mast cell secretory granules [18]. Small amounts of tryptase are found in basophils also. When tryptase is released, it is secreted as an active proteoglycan complex with a large size (200 to 250 kDa) that limits diffusion away from

mast cell activation sites. Therefore, circulating tryptase levels increase only after the massive mast cell activation seen in anaphylactic shock or mastocytosis. Tryptase can spread the degranulation signal from mast cell to mast cell [19]. Serum tryptase peaks 60 to 90 minutes after the onset of anaphylaxis and remains elevated for up to 5 hours [20], making this an effective laboratory marker for anaphylaxis. There are α , β , and γ tryptases, and only minor differences in amino acid sequences are seen between α and β . α -tryptase is thought to be released constitutively from mast cells in an inactive form and the stored forms of tryptase which are released during degranulation thought to be β -tryptase [21]. The study by Schwartz and colleagues [22], however, suggests that pro- α and β tryptases, unprocessed tryptases before propeptide removal, are secreted constitutively rather than stored granules with mature tryptases. Mature β -tryptase level is elevated only after severe anaphylaxis, and it is shown in Fig. 3 [23]. The ratio of total tryptase to β -tryptase is helpful in differentiating anaphylaxis from mastocytosis (Table 1). A ratio of 10 or less is indicative of anaphylaxis not associated with systemic mastocytosis, and a ratio of 20 or greater indicates systemic mastocytosis [23,24]. This is helpful, especially when anaphylaxis occurs in a systemic mastocytosis patient who has a high baseline total tryptase level [25]. Increased level of total tryptase often correlates with an increased histamine level, but imperfect correlation may be seen, because β -tryptase diffuses through tissue more slowly than histamine with its association with the protease/proteoglycan complex [23] and also probably

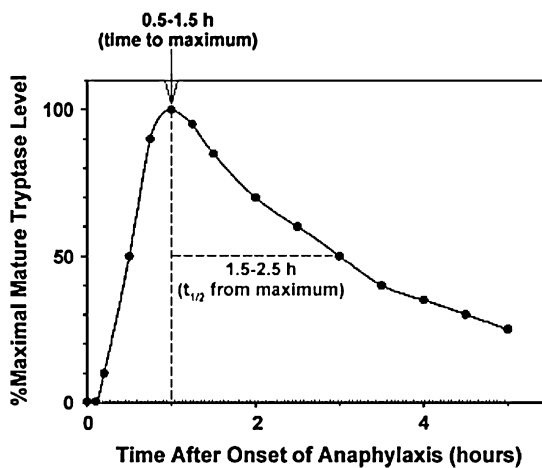


Fig. 3. Hypothetic time course for the appearance of mature tryptase in serum or plasma during systemic anaphylaxis. The maximal level is set at 100% in the figure; however, in reality, it varies depending at least in part on the clinical severity and nature of the anaphylactic stimulus, which, in turn, affects how long mature tryptase is in a detectable range. *Abbreviation:* $t_{1/2}$, half-life. (From Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin N Am* 2006;26:451–63; with permission.)

Table 1
Mature and total tryptase levels

Clinical condition	Tryptase levels (ng/mL)		Tryptase ratio (total/mature)
	Total	Mature	
Normal	1–15	< 1	Not applicable
Systemic anaphylaxis (acute)	Greater than baseline	> 1 ^a	< 10
Systemic mastocytosis (nonacute)	> 20 ^b	< 1 to small elevations	> 20

^a Level related to clinical severity (hypotension), timing of sample collection in relation to onset of signs and symptoms, and nature of the anaphylactic stimulus.

^b Speculated to reflect primarily the total body burden of mast cells.

Data from Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin N Am* 2006;26:451–63.

because of difference in time course. Tryptase level is more likely to elevate in severe anaphylaxis, and a normal tryptase level does not rule out anaphylaxis.

As seen in [Box 1](#) [10], multiple inflammatory cascades (contact system, clotting system, and complement system) are involved in anaphylactic events. Along with tryptase, mast cell kininogenase and basophil kallikrein activate these systems. Tryptase can activate the contact (kallikrein-kinin) system, which leads to decreased high molecular weight kininogen and formation of activation complexes, followed by bradykinin production, causing angioedema. Tryptase also can inactivate procoagulant proteins [26] and promote fibrin clot lysis [27], which may lead to disseminated intravascular coagulation.

Box 1. Multimediator recruitment occurring during anaphylaxis and anaphylactic events (pathway activated)

Coagulation pathway

- Decreased factor V
- Decreased factor VIII
- Decreased fibrinogen

Complement cascade

- Decreased C4
- Decreased C3
- Formation of C3a

Contact system (kinin formation)

- Decreased high-molecular-weight kininogen
- Formation of kallikrein-C1-inhibitor complexes and factor XIIa-C1-inhibitor complexes

Chymase

As seen with tryptase, chymase also is bound to proteoglycans and stored in mast cell granules [2]. Chymase is a monomer and present only in MC_{TC} mast cells. Chymase can activate the angiotensin system, with conversion of angiotensin I to angiotensin II to compensate intravascular volume loss secondary to increased vascular permeability [10]. It has been suggested by Hermann and colleagues [28,29] that baseline angiotensin II levels and I are lower in hymenoptera-sensitive patients than normal patients, and the severity of the anaphylaxis correlates with lower levels. Hence, intake of angiotensin-converting enzyme (ACE) inhibitors may predispose anaphylaxis because of blockage of this compensatory effect, and it is beneficial to consider avoiding ACE inhibitors in the setting of chronic anaphylaxis or immunotherapy. Chymase also cleaves neuropeptides, such as vasoactive intestinal peptide, and acts on substance P and bradykinin.

Proteoglycans—heparin

As discussed previously, human mast cells contain heparin and chondroitin sulfate proteoglycans to stabilize secretory granule proteases [2]. When mast cells are activated, heparin is released from mast cell granules with proteases and histamine. Heparin can work as anticoagulation by binding to antithrombin III, so that clotting cascade will not progress. Heparin also is known to bind phospholipase A2 and inhibit the arachidonic acid cascade, which yields factors that are chemoattractants for eosinophils. Heparin also inhibits cytokine function, activation of kinin pathway, and the complement cascade.

Membrane-derived lipid mediators

Mast cells generate and release eicosanoids lipid mediators such as prostaglandins and leukotrienes through multiple enzymatic steps from nuclear membrane phospholipids. Arachidonic acid (AA) is cleaved from membrane phospholipids by means of cytosolic phospholipase A2, and subsequently metabolized by several routes, including the cyclooxygenase pathway and 5-lipoxygenase pathway. These pathways are illustrated in Fig. 4. After FcεRI activates bone marrow mast cells, two phases of eicosanoid lipid mediator generation are seen [2]. An acute phase takes place within 10 minutes, and leukotriene C (LTC)4 and prostaglandin D (PGD)2 are generated dependent on constitutively expressed cyclooxygenase (COX)-1. During a delayed phase (2 to 10 hours after activation), PGD2 alone is synthesized by induced COX-2.

Prostaglandins

COX-1 and COX-2 are enzymes that catalyze conversion of arachidonic acid to prostaglandins (PGs) [2]. The major COX product is PGD2 in mast cells. Basophils do not produce PG2, making this a distinguishing feature

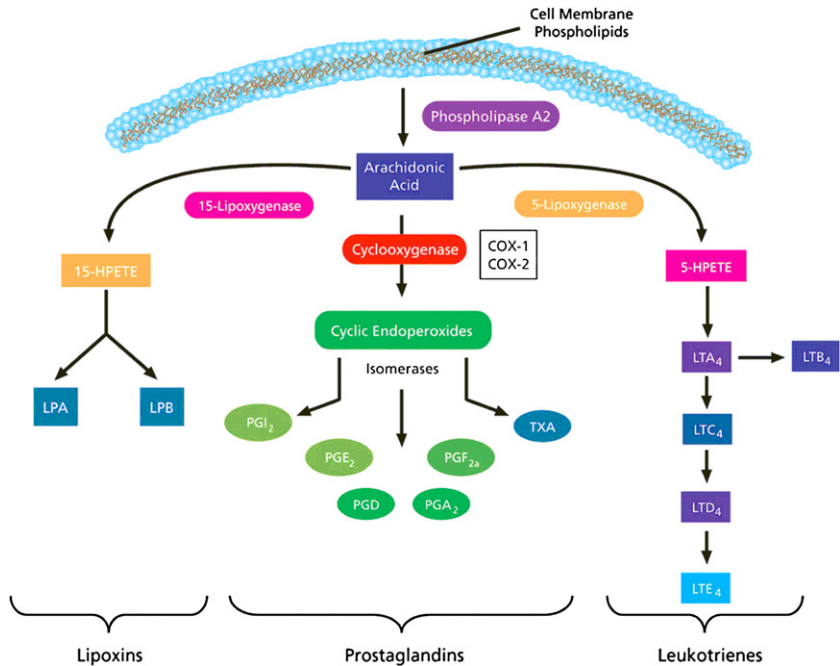


Fig. 4. Arachidonic acid cascade. (Courtesy of Sigma-Aldrich Co., St. Louis, MO; with permission. © 2005 Sigma-Aldrich Co.)

from mast cells. After synthesis in mast cells, PGD₂ is excreted by means of a prostaglandin transporter protein and binds to two receptors DP1 and DP2. DP2 receptors are found in most tissues. PGD₂ produces bronchoconstriction, peripheral vasodilation, and coronary and pulmonary artery vasoconstriction. Operating through the DP2 receptor, PGD₂ is chemotactic for basophils, eosinophils, dendritic cells, and Th2 cells, and it is an enhancer of histamine release from basophils. Skin mast cells mainly produce PGD₂, whereas mast cells at the lung, heart, and GI tract secrete predominately PGD₂ and LTC₄.

PGF₂ is also a bronchoconstrictor, peripheral vasodilator, coronary vasoconstrictor, and platelet aggregation inhibitor. PGE₂ is mainly a bronchodilator, but the rest of the PGs are bronchoconstrictors. Thromboxane A₂ causes vasoconstriction, platelet aggregation, and bronchoconstriction.

Leukotrienes

Cysteinyl leukotrienes (cysLTs) are a class of structurally related lipid molecules, originally described as slow-reacting substance of anaphylaxis, with a myriad of biologic functions [2,30,31]. CysLTs are synthesized by mast cells, basophils, and eosinophils. LTC₄ is formed when LTC₄ synthase conjugates glutathione to leukotriene A (LTA)₄. After cellular export, LTC₄ is metabolized to LTD₄ and LTE₄ by sequential removal of glutamic

acid and glycine. The biological activities of the cysLTs include producing smooth muscle contraction and mucus secretion, recruiting allergic inflammatory cells, modulating cytokine production, influencing neural transmission, and altering structural changes in the airway. CysLTs potentially can stimulate smooth muscle contraction independent of histamine. On inhalation, LTC₄ and LTD₄ induce bronchoconstriction in normal subjects with a 1000-fold greater potency than histamine, and LTE₄ is 10-fold more potent [32]. CysLTs also cause increased vascular permeability, which can induce wheal-and-flare responses in skin, and cause arteriolar constriction. Denzlinger and colleagues [33] investigated cysteinyl leukotriene production in anaphylactic reactions and found that the concentrations of urinary leukotriene E₄ plus N-acetyl leukotriene E₄ were enhanced strongly in urine samples during or shortly after the anaphylactic reaction. Correlation between leukotrienes and anaphylaxis is suggested especially in exercise-induced anaphylaxis in animal models [34]. LTB₄ is a chemotactic agent and thus theoretically might contribute to the late phase of anaphylaxis and to protracted reactions [35].

There are two receptors for the CysLTs, CysLT₁ and CysLT₂. Both are G protein coupled transmembrane receptors. CysLT₁ is expressed highly on lung smooth muscle cells, peripheral blood leukocytes including eosinophils and monocytes, and on mast cells. CysLT₁ antagonists, including montelukast, are effective in attenuating early and late responses of allergen exposures, chronic bronchial asthma, and exercise challenges, but no clinical studies have shown the effect of any antileukotrienes to modify the course of anaphylaxis.

Platelet-activating factor

Platelet-activating factor (PAF) is synthesized from membrane phospholipids by means of a different pathway from arachidonic acid. It is produced by acetylation at the SN-2 position after PLA₂ cleavage of AA in the membrane, and binds to a G protein-coupled 7 transmembrane receptor. PAF aggregates platelets (that is how PAF originally was named), and it is also a very potent mediator in allergic reactions and stimulates a broad range of cell types. PAF causes bronchoconstriction with the potency of 1000 times greater than histamine, but also increases vascular permeability, chemotaxis, and degranulation of eosinophils and neutrophils.

Cauwels and colleagues [36] recently showed a surprising finding that hyperacute PAF shock depends entirely on NO, produced by constitutive eNOS, instead of iNOS. In this murine model, PAF was administered to wild-type, iNOS knockout, and eNOS knockout mice. Only eNOS knockout mice survived. One plausible explanation is that anaphylaxis overstimulates eNOS and thereby excessively relaxes vascular smooth muscle, leading to hypotension and death. eNOS is phosphorylated rapidly and activated by means of the PI3 K pathway in endothelial cells, which might lead to excessive NO synthesis.

Cytokines and chemokines

Mast cells release chemokines and cytokines, as well as previously mentioned mediators, and contribute to anaphylactic reactions. Chemokines and cytokines mainly contribute to the late phase of biphasic anaphylactic reaction, seen several hours after the first symptoms subside. TNF- α is the major mast cell inflammatory cytokine. After IgE-, IgG-, and toll-like receptor-mediated activation of mast cells, preformed TNF- α is released, and it activates neutrophils, increases monocyte chemotaxis, and enhances production of other cytokines by T cells. Anti-TNF treatment theoretically improves anaphylaxis, but no clinical trials or animal studies have been reported.

After mast cells are activated by means of Fc ϵ RI, expression of nuclear factor (NF)- κ B related genes, CCL-7, CCL-1, and Th2 cytokine genes (interleukin [IL]-5, IL-13) are enhanced. Other cytokines released include granulocyte (G) colony-stimulating factor (CSF), macrophage (M)-CSF, GM-CSF, IL-1 beta, IL-3, IL-4, IL-6, IL-8, IL-10, IL-16, IL-18, IL-22, and TNF- α [2]. Basophils are a major source of IL-4, IL-13, and chemokines [37]. In anaphylaxis, when Fc ϵ RI-mediated activation takes place in the gut, lung, and skin, a chemokine receptor CCR3 is expressed predominantly on MC_{TC}. After CCR3 is translocated to the surface membrane, the mast cell becomes susceptible to its ligand CCL11 (eotaxin), and CCL 11 binds to CCR3, leading to increased secretion of IL-13.

IL-4 stimulates and maintains Th2 cell proliferation and switches B cells to IgE synthesis. IL-4 and IL-13 increase IgE production, Fc ϵ RI expression, and target cell responsiveness to various vasoactive mediators. IL-4/IL-13 activation of the transcription factor signal transducer and activator of transcription 6 plays a key role by means of IL-4 receptor α chain [38]. Therefore, IL-4 R α antagonists might be an effective treatment for anaphylaxis, particularly as a prophylactic therapy. Although long-term exposure to IL-4 stimulates mastocytosis and can enhance mediator release, the most dramatic and rapid effect of IL-4 on anaphylaxis is a three- to sixfold enhancement of responsiveness of targeted cells to vasoactive mediators, including histamine, serotonin, PAF, and cysteinyl leukotrienes (Strait RT, Morris SC, Smiley K, Urban JF Jr, Finkelman FD, unpublished data, 2005). These effects were blocked by IFN- γ , a cytokine that inhibits many effects of TH2 cytokines [39–41].

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