Anaphylaxis: Mediators, Biomarkers, and Microenvironments

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Abstract

The life-threatening nature of anaphylactic reactions has increased interest in discovering new biomarkers that could improve diagnosis and prevention. However, the diverse nature of the clinical features and the etiology and pathogenesis of anaphylaxis hinder the identification of valuable molecular indicators of disease.

Most studies on anaphylaxis focus on the immune system. Anaphylactic reactions are characterized primarily by IgE-mediated activation of mast cells and basophils and release of mediators. Determination of serum tryptase levels is the main in vitro test used to confirm the reaction, although there are no biomarkers that can predict it. Nevertheless, recent research has postulated that alternative pathways, cell types, and systems are involved. Consequently, various molecular products have been explored and considered potential biomarkers, although none of them are yet used in clinical practice. The products that are altered in patients with anaphylaxis include vasoactive agents, proteases, proteoglycans, lipids, interleukins, cytokines, products of the complement-contact and coagulation systems, circulating proteins, extracellular vesicles, microRNAs, and metabolites. The recognition of biological processes and molecular pathways affecting the microenvironments involved in anaphylaxis will considerably improve clinical practice and the identification of better molecular markers. We offer a broad review of the various mediators described in anaphylaxis, consider their usefulness as potential biomarkers of this pathological event, and examine their role in the molecular basis of the reaction.

Key words: Anaphylaxis. Biomarker. Mast cells. Mediators. Microenvironments.

Resumen

La naturaleza potencialmente mortal de las reacciones anafilácticas promueve un creciente interés por descubrir nuevos biomarcadores que puedan ayudar a su diagnóstico y prevención. Sin embargo, tanto las características clínicas como la etiopatología de la anafilaxia son muy diversas, lo que dificulta la identificación de marcadores moleculares precisos.

La mayoría de los estudios sobre la anafilaxia se han centrado en el sistema inmunitario. En concreto, estas reacciones se caracterizan principalmente por la activación de mastocitos y basófilos mediada por IgE y la liberación de diferentes mediadores. Entre ellos, la determinación de los niveles de triptasa sérica es la principal prueba *in vitro* utilizada para confirmar la reacción y no existen biomarcadores con capacidad predictiva. Sin embargo, estudios recientes han postulado que existen otros tipos celulares, sistemas y vías de señalización alternativos implicados en la anafilaxia. En consecuencia, se han explorado e identificado diferentes moléculas como potenciales biomarcadores, pero ninguno de ellos se ha trasladado aún a la práctica clínica. Precisamente, los agentes vasoactivos, las proteasas, los proteoglicanos, los derivados lipídicos, las interleuquinas, las citoquinas, los productos de los sistemas de complemento-contacto y de coagulación, las proteínas circulantes, las vesículas extracelulares, los microARN y los metabolitos, se encuentran alterados en pacientes con anafilaxia. El reconocimiento de los procesos biológicos y de las vías moleculares que interactúan en la magnitud de los microambientes implicados en la anafilaxia mejorará notablemente la práctica clínica y el reconocimiento de marcadores moleculares. Por lo tanto, este artículo abarca una amplia revisión de los distintos mediadores descritos en la anafilaxia y de su propuesta como biomarcadores de este evento patológico, así como su implicación en las bases moleculares de la reacción.

Palabras clave: Biomarcador. Mastocitos. Mediadores. Microambientes.

Introduction

Anaphylaxis is an acute systemic reaction and the most severe manifestation of allergic disorders. The incidence of this pathological event is underdiagnosed owing to the lack of effective biomarkers. The main molecular marker used in clinical practice is serum tryptase (sT), which is not elevated in most cases. In this regard, anaphylactic reactions are classified into different phenotypes and endotypes, with mast cells (MCs) and basophils as the main releasers of this biomarker. However, other molecular and cellular components have also been characterized in human anaphylaxis [1].

The term *biomarker* includes any biological observation that replicates or predicts a clinically relevant endpoint. However, it can also serve to predict diseases, to identify cells involved in their etiology and pathogenesis, and to assess an individual's response to treatment. In clinical practice, a biomarker is usually a mediator involved in the cellular basis of the disease [2]. Recent key statements in anaphylaxis point to the necessity to identify reliable diagnostic, predictive, and prognostic biomarkers [3]. Therefore, the aims of this review are to delve into the different molecules altered during anaphylaxis and to determine their role in the microenvironments involved in the reaction.

1. Anaphylaxis

The World Allergy Organization defines anaphylaxis as follows: "Anaphylaxis is a serious systemic hypersensitivity reaction that is usually rapid in onset and may cause death. Severe anaphylaxis is characterized by potentially life-threatening compromise in airway, breathing and/or the circulation, and may occur without typical skin features or circulatory shock being present" [4]. The most common signs and symptoms are observed in the skin and mucous membranes (80-90%), followed by the respiratory system (70%). Less common, however, is the involvement of the gastrointestinal (45%), cardiovascular (45%), and nervous systems (15%) [5,6].

The incidence and prevalence of anaphylaxis has increased worldwide over the last 2 decades [7-10]. This pathological event can be caused by various substances, with food and drugs being the most common triggers. Foods are the most frequent in children, while drugs are more prevalent in adults [11]. However, epidemiological data are underestimated because of the absence of biomarkers and the ambiguity of the diagnosis of anaphylaxis [4,5].

Diagnosis is based on the recognition of signs and symptoms [4,12]. Moreover, it could be complemented by measurement of sT, the main biomarker used in clinical practice [4]. Over the years, many studies have reported this molecule to be increased in patients' sera [13-17]. sT peaks appear from 15 minutes after the onset of reaction symptoms and last for several hours [18,19]. However, levels do not always correlate with the severity of anaphylaxis and remain low in 36%-50% of events [14,20], especially in mild cases, where the reaction is not well diagnosed clinically [20].

Despite the wide variability in sT values, the clinical threshold for this protein in anaphylaxis is set at ≥11.4 ng/mL [14,21]. Nevertheless, there have also been reports of cases of MC activation with sT levels below this cut-off point [15,22,23]. Therefore, sT should be assessed at least 24 hours after the episode [4,19]. Specifically, the difference between the acute peak of this protein and its baseline value was greater in anaphylaxis than in other diseases related to MC activation [19]. Accordingly, other sT thresholds proposed for the diagnosis of anaphylaxis include an increase of 20%+2 ng/mL in the acute condition compared to baseline [15,19,24]. In addition, a study carried out in Hymenoptera venom–allergic patients defined MC activation as acute sT values ≥135% relative to baseline [22]. One recent study points to an improved diagnostic capacity of tryptase when personalized medicine is applied based on the patient's baseline status [25].

2. Pathophysiology of Anaphylaxis

Anaphylaxis is an acute systemic reaction involving several organs and systems. It is caused by the simultaneous activation of multiple molecular mechanisms and the sudden release of mediators, giving rise to signs and symptoms [26]. For many years, studies have focused on the analysis of the immune component, although the importance of other altered systems in etiology and pathogenesis has been demonstrated [27,28]. Other, as yet unexplored mechanisms likely contribute to the release of these or other mediators (Table). Therefore, understanding the plethora of specific microenvironments is essential when attempting to identify appropriate biomarkers and apply appropriate management based on precision medicine [29].

2.1 Immune System

Anaphylaxis has traditionally been considered a type I hypersensitivity reaction based on an IgE-mediated immunological mechanism. When it occurs, mediators are released from activated MCs and basophils, thus inducing the signs and symptoms of this pathological event [18]. However, degranulation of these cells can be achieved by different surface receptors and mechanisms depending on the nature of the stimulus (Figure 1) [30,31].

IgE-mediated reactions are divided into 2 phases: a sensitization phase and a re-exposure/activation phase. In primary sensitization, allergens induce the release of IgE into the bloodstream. This then binds to the high-affinity receptor for IgE (FcεRI) on the surface of MCs and basophils [32,33]. However, symptoms appear after a new exposure to the allergen, when activation of MCs and basophils and sudden release of mediators occur [34,35].

However, some patients experience anaphylaxis with undetectable allergen-specific IgE levels according to skin or in vitro testing. Furthermore, elevated levels of allergen-specific IgE have been found in patients who have not experienced a reaction [34,36-38]. Therefore, these events point to the presence of other processes and cells involved in the reaction.

IgG-mediated anaphylaxis was first characterized in mouse models [39,40]. This pathway has since been described in humans and could also be responsible for numerous episodes of IgE-independent reactions, although the number of studies

Table. Mediators Released From Activated Cells and Systems in Anaphylaxis

		MC	B	N	MON	MAC	LT	EOS	PLAT	EC	VSMC	N	E	P
PROTEASES	Tryptase	✓	✓											
	Carboxypeptidase A	✓	✓											
	Chymase	✓												
	Elastase	✓	✓	✓										
	MPO			✓	✓	✓								
VASOACTIVE AGENTS	Histamine	✓	✓	✓										
	Bradykinin													✓
	Nitric Oxide									✓				
	Endothelin-1				✓	✓				✓	✓			
	VEGF	✓			✓		✓		✓	✓	✓			
LIPIDIC MOLECULES	LTB; C, D, E 4	✓	✓	✓										
	PGD2	✓	✓		✓	✓			✓					
	PGE2	✓	✓			✓		✓	✓		✓			
	PGF2	✓							✓					
	TXA2	✓						✓	✓					
	TXB2	✓												
	PAF	✓	✓	✓	✓	✓		✓	✓	✓				
	S1P	✓						✓		✓	✓		✓	

Fcε Receptor I
Fcε Receptor II
Fcγ Receptor
Mas-Related G Protein-Coupled Receptor

		MC	B	N	MON	MAC	TL	EOS	PLAT	EC	VSMC	N	E	P
HOMEOSTATIC FACTORS	C/C/C													✓
	Heparin	✓	✓											
	RAS													✓
	Apolipoproteins													✓
	Haemoglobin												✓	
NERVOUS SYSTEM	SP											✓		
	CGRP											✓		
	Serotonin	✓							✓			✓		
	Adenosine	✓												
	Nor/epinephrine											✓		
CYT & CHEM	TNF-α*1	✓	✓	✓		✓								
	TGF-β	✓						✓						
	IFN-γ			✓			✓							
	GM-CSF	✓	✓											
	CCL-2	✓		✓	✓	✓				✓				
	PF4								✓					
INTERLEUKINS	IL-2						✓							
	IL-4	✓	✓				✓	✓						
	IL-5	✓					✓	✓						
	IL-6	✓	✓	✓		✓								
	IL-10	✓						✓						
	IL-13	✓	✓					✓						
	IL-33	✓	✓											

Fcε Receptor I
Fcε Receptor II
Fcγ Receptor
Mas-Related G Protein-Coupled Receptor

Abbreviations: B, basophil; C/C/C, contact, coagulation, and complement systems; CCL-2, C-C motif chemokine ligand 2; E, erythrocyte; EC, endothelial cell; EOS, eosinophil; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; IL, interleukin; LTB; C, D, E4, leukotrienes B, C, D, E4; MAC, macrophage; MC, mast cell; M-CSF, macrophage colony-stimulating factor; MON, monocyte; MPO, myeloperoxidase N, neutrophil; TL, T lymphocyte; PAF, platelet-activating factor; PF4, platelet factor 4; PGD2, prostaglandin D2; PGE2, prostaglandin E2; PGF2, prostaglandin F2; PLAT, platelet; T lymphocyte; RAS, renin-angiotensin system; SP, substance P; TGF-β, transforming growth factor beta; TNF, tumor necrosis factor (*1 TNFα members family including TNF-α, TNFRI, and TWEAK); VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell; P, plasma; S1P, sphingosine-1-phosphate; TXA, thromboxane A2; TXB2, thromboxane B2.

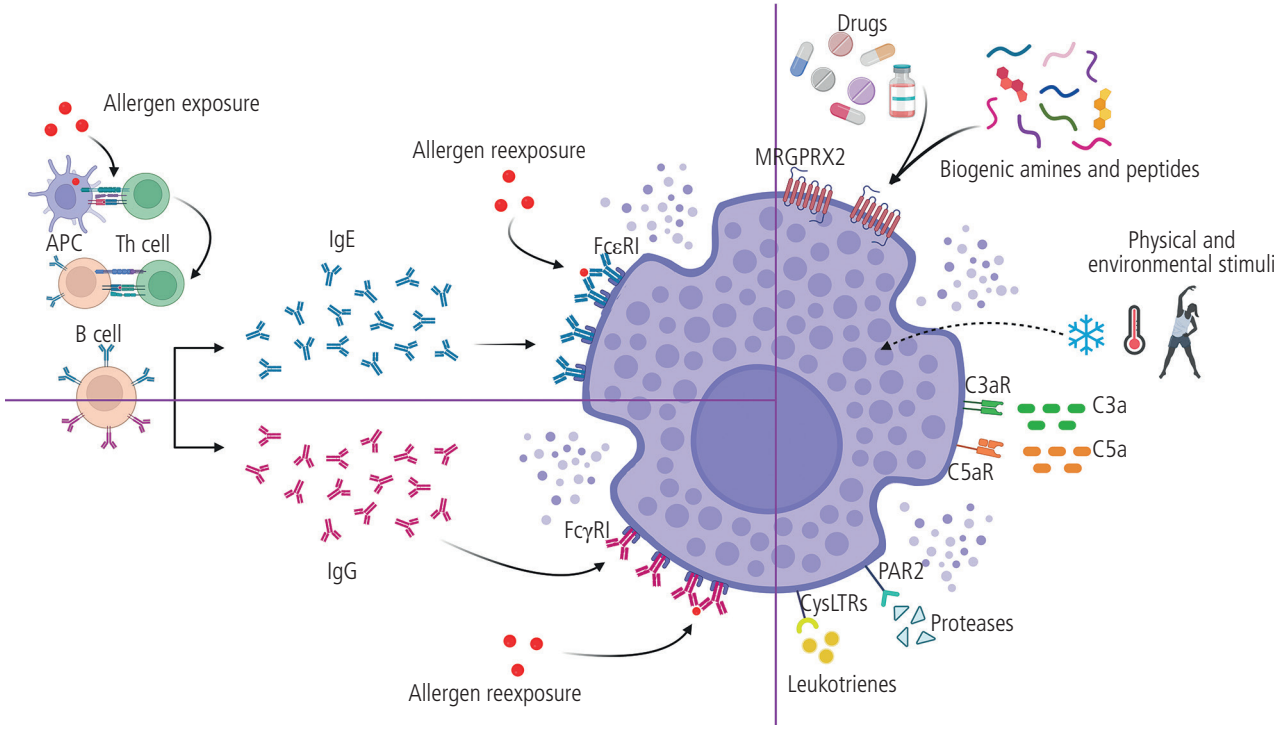

Figure 1. MCs are activated through multiple molecular mechanisms in anaphylaxis. Classically, MCs can be activated through cross-linking of FcεRI (upper left panel). They can also respond to IgG immune complexes (lower left panel). Other ligands and/or signals activate MCs through receptors such as MRGPRX2, complement receptors, and many other receptors. APC indicates antigen-presenting cell; TH cell, helper T lymphocytes; B cell, B lymphocytes; FcεRI, Fc epsilon receptor I; FcγRI, Fc gamma receptor I; MRGPRX2, Mas-related G-protein coupled receptor member X2; C3a, complement component 3a; C5a, complement component 5a; C3aR, complement component 3a receptor; C5aR, complement component 5a receptor; PAR2, protease activated receptor 2; CysLTRs, cysteinyl leukotriene receptors.

in humans remains limited [41-43]. IgG antibodies act through binding to Fc gamma receptors (FcγRs), which are expressed in various cell types such as macrophages, dendritic cells, neutrophils, platelets, basophils, and MCs [43-46]. Specifically, neutrophils are thought to be the main cells involved in IgG-mediated human anaphylaxis [41,42,44]. They are activated by the formation of IgG–allergen immune complexes and the subsequent binding to FcγR receptors on their surface, leading to the release of many protein and lipid mediators [35,36,43]. These include platelet activating factor (PAF), which is one of the main molecules stored in neutrophils and whose role in the pathogenesis of anaphylaxis has been demonstrated both in mice and humans [43,47].

2.1.1 Anaphylactic mediators released by immune cells

In anaphylaxis, the release of molecules previously stored in granules or newly synthesized molecules has traditionally been understood to be derived from MCs and basophils. However, the discovery of other cell types and the subsequent release of other mediators show anaphylactic reactions to be heterogeneous in nature. Among the many molecules released, tryptase is the main biomarker in clinical practice, although many others have been proposed.

2.1.1.1 Histamine

Histamine is a diamine prestored in granules of MCs and basophils and is considered, together with tryptase, one of the main mediators of anaphylactic reactions [48,49]. The mechanism of action of this vasoactive peptide is through 4 receptors (H1, H2, H3, and H4), which are expressed in numerous cell types [49-51]. Their activation gives rise to multiple intracellular signalling pathways, thus inducing many of the symptoms of anaphylaxis, such as increased vascular dilatation and permeability, as well as constriction of the airways [34,52,53].

In anaphylaxis, the increase in plasma histamine levels shows them to be a promising biomarker [13,54,55]. A study of 97 patients visiting the emergency department shows that almost half of them present elevated serum histamine levels. Likewise, another study of 76 patients found that molecule levels are significantly higher in severe reactions than in moderate reactions [54,55]. Moreover, detection of histamine in body fluids is simple, and some studies have even proposed it as a more sensitive diagnostic molecule than tryptase. However, its use as a potential diagnostic marker has several limitations [55,56], including its short half-life (approximately 30 minutes), peaking 5-10 minutes after initiation of the reaction, thus hampering sample collection [56,57].

2.1.1.2 Enzymes: proteases and peroxidases

Among the proteases released by the degranulation of effector cells, sT is the most relevant and easily detectable molecule in blood samples from patients with anaphylaxis.

However, other proteases have also been considered candidate biomarkers [36].

Carboxypeptidase A is a zinc-dependent metalloprotein that can also be found as a preformed mediator in basophils [56,58,59]. The levels of this protein are elevated in the serum of patients with anaphylaxis and in those who die from drug-induced reactions [60]. However, their levels do not necessarily correlate with those of sT and may even be increased in cases where sT levels remain low [36].

Chymase is a serine proteinase whose biological effects include activation of the renin angiotensin system. Chymase activity is heparin-dependent, and levels are elevated in the serum of anaphylactic patients up to 24 hours after onset of the reaction [61,62]. In addition, a study of patients who died of anaphylaxis showed not only increased chymase levels, but also that their value correlated with those of sT [63]. Moreover, the renin-angiotensin system is the main homeostatic regulator of blood pressure, contributing to other processes, such as proliferation, fibrosis, and inflammation [64]. One study of 50 patients with anaphylaxis due to hymenoptera venom showed that renin, angiotensinogen, angiotensin I, and angiotensin II levels decreased significantly and in accordance with the severity of the reaction [65].

Elastase is a serine protease released mainly by neutrophils, although it may also be present in the granules of basophils and MCs [66]. This protein can cleave the kinin light chain activating the contact system [61,67]. Furthermore, myeloperoxidase is a potent oxidizing enzyme found in neutrophil azurophilic granules. Neutrophil extracellular traps, which are detected as DNA-myeloperoxidase complexes together with elastase, have been described as increased during human anaphylaxis in line with the severity of the episodes [41,43,47].

2.1.1.3 Lipid molecules

Bioactive lipids are involved in immune function and vascular biology. Consequently, their relevance in allergic diseases and anaphylaxis has also been recognized, both in terms of the underlying molecular basis and as a complementary biomarker of sT [68,69].

PAF is a biologically active phospholipid that induces platelet aggregation and plays key roles in cardiovascular pathophysiology [70]. This mediator is released by multiple cell types and is involved in processes such as inflammation, proliferation, and cell adhesion [71,72]. In anaphylaxis, PAF induces the release of several cytokines and participates in bronchoconstriction, hypotension, and endothelial permeability [73]. In addition, its elevation is associated with hemodynamic changes that occur in severe cases, including alterations in right ventricular pressure and total pulmonary resistance [44]. Consequently, this molecule has been proposed as a potential therapeutic target in this pathological event [53]. PAF is one of the most widely evaluated mediators as a biomarker in anaphylaxis, although it has not been extrapolated to clinical practice. Studies carried out by Vadas et al [16,74] revealed that PAF increased with the severity of the reaction in the plasma of adults and children. Furthermore, a higher percentage of patients were diagnosed based on PAF than with other biomarkers, such as histamine and tryptase [16]. However, this mediator has a short half-life because it is rapidly inactivated, thus hampering its application in clinical practice, since patients with anaphylaxis often attend the emergency department within minutes or hours of the onset of symptoms [74]. The main enzyme involved in degradation of PAF is PAF acetylhydrolase [75,76], which has also been proposed as a possible molecular marker for anaphylaxis, with various studies suggesting that its levels drop during the reaction [17,74,77].

Sphingosine-1-phosphate (S1P) is a bioactive lipid involved in a broad spectrum of cellular processes and is supplied to plasma from MCs, erythrocytes, platelets, and endothelial and smooth muscle cells [78]. It plays a key role in the signaling of vascular homeostasis and allergic disorders [68]. In addition, the balance between intracellular and extracellular S1P and their functional receptors attributes relevant features to several human inflammatory and allergic responses [79,80]. Intracellular levels of this molecule can be regulated by sphingosine kinases and are essential for maintaining endothelial barrier function [81]. In vivo systemic anaphylactic response has been associated with rapid depletion of circulating S1P concentrations [78,82].

Arachidonic acid–derived lipid molecules are synthesized in anaphylaxis and grouped into families including prostaglandins (PGE2, PGD2, PGF2α), prostacyclins (PGI2), cysteinyl-leukotrienes (LTC4, LTD4, and LTE4), and thromboxanes (TXA2) [83,84]. These molecules are derived from membrane bilayers through action of cyclooxygenase or the lipoxygenase pathway. Early studies in anaphylaxis observed the presence of slow-reacting substances with biologic activity after antigenic stimulation on sensitized tissues [85]. Years later, these substances are considered a blend of prostaglandins and cysteinyl-leukotrienes released mainly through MC activation [35,86]. In addition, some of the molecules are considered valuable biomarkers in anaphylaxis [84,87,88]. Interestingly, nonsteroidal anti-inflammatory drugs have been associated with increased PGE2 and the production and overexpression of adenosine receptor 3 [89,90].

2.1.1.4 Cytokines and chemokines

Cytokines are released by a wide variety of cells, although the main contributors are those of the immune system. These proteins present a diversity of functions, play a key role in immune responses, and are mainly involved in inflammatory processes [91,92]. In anaphylaxis, increased levels of cytokines have been observed in the most severe cases [17].

Tumor necrosis factor α (TNF-α) is one of the most studied cytokines. During anaphylaxis, it is released mainly by the activation and degranulation of MCs [93]. Indeed, increased levels of TNF-α have been described in experimental models and in serum samples of patients with anaphylaxis [54,94,95]. In turn, increased values of its receptor, TNF receptor I (TNFRI), have also been observed in most cases of severe anaphylaxis [17,54]. Accordingly, circulating levels of TNF-like weak inducer of apoptosis and of its receptor (Fn14) have been shown to increase in patients with anaphylaxis. These proteins play a crucial role in the alterations in vascular permeability underlying this pathological event [94].

Tumor growth factor β (TGF-β) is produced mainly by activated MCs and has an inhibitory effect on the anaphylactic

reaction by blocking the release of TNF-α [93,96]. TGF-β is increased in the serum of patients with anaphylaxis [97].

Interferon-γ (IFN-γ) is a proinflammatory cytokine that plays a key role in the T_H1 response [98]. In anaphylaxis, peripheral blood mononuclear cells from patients have been shown to release higher levels of this protein [99]. In addition, elevated values of IFN-γ have been observed in the serum of patients with anaphylaxis [54].

Granulocyte macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor that is increased in patients with anaphylaxis. GM-CSF modulates the maturation of several immune and inflammatory cells, including the main effector cells of the reaction [54]. In addition, it plays a role in inflammation by mediating communication between tissues and immune cells [100].

Platelet factor 4 is released by platelets following activation of the coagulation system [101], and its levels have been found to be increased in rabbit anaphylactic models [102].

Chemokines play a role in attracting and guiding various families of leukocytes [103], including CCL-2, also known as monocyte chemoattractant protein 1, which is increased in mice models and human anaphylactic samples [104-106]. In addition, a study conducted in serum from pediatric patients with anaphylaxis also demonstrated increased values for this chemokine and CCL-11 [107].

2.1.1.5 Interleukins

The term interleukin (IL) was coined to describe cytokines produced by leukocytes that play the role of messengers in different parts of the immune system [108]. However, these mediators can be produced by many other cell types (including resident cells) and exert pleiotropic effects [109,110].

Serum levels of IL-2, IL-4, IL-5, IL-6, IL-10, and IL-13 are increased in patients with severe anaphylaxis [54]. IL-2, IL-6, and IL-10 correlate with the severity of the episodes, histamine concentration, and hypotension [54,106,111]. Specifically, mouse anaphylactic models show that IL-6 is released within the first few hours after allergen exposure [112]. In humans, high levels of IL-6 have been observed in hypersensitivity reactions and anaphylaxis induced by monoclonal antibodies, as well as in perioperative patients [106,113]. IL-33 promotes oral anaphylaxis after epicutaneous sensitization in experimental murine models [114].

2.1.2 Complement and contact/coagulation systems

Complement and contact/coagulation systems are involved in the pathophysiology of anaphylaxis [61,115]. These molecular cascades are in constant crosstalk and in continuous amplification and inhibition loops, thus keeping their effect balanced during inflammation. For example, components of the coagulation system amplify complement activation, which, in turn, magnifies coagulation and inhibits fibrinolysis [116]. Factors and molecules derived from their proteolytic processes are altered in anaphylaxis, thus indicating not only a potential function of these systems in hypersensitivity reactions, but also the possibility of evaluating them as candidate diagnostic biomarkers.

2.1.2.1 Complement system

The complement system is composed of a complex cascade of proteases that participate in both the innate and the adaptive immune responses through the activation of 3 distinguishable pathways: the classical, the alternative, and the lectin pathways [117].

In anaphylaxis, complement seems to be activated mostly by the classical pathway through the formation of IgG immunocomplexes. This process leads to the release of C3a, C4a, and C5a from the proteolysis of the C3, C4, and C5 components [117,118]. These soluble products, also known as anaphylatoxins, bind to specific G protein–coupled receptors in MCs and basophils and thereby stimulate their degranulation [119-121]. Indeed, levels of C3a, C4a, and C5a have been found to be elevated in the sera of patients with anaphylaxis and seem to be correlated with the severity of the reaction [17]. In addition, there is clinical evidence demonstrating the induction of this type of hypersensitivity reaction by agents that directly activate the complement system, which would confirm that anaphylatoxins alone would be capable of triggering the reaction [122].

2.1.2.2 Contact/coagulation systems

The contact system consists of a series of proteases and coagulation factors that are activated in response to inflammatory macromolecular complexes or negatively charged surfaces. In turn, it enables formation of bradykinin and the activation of the intrinsic coagulation cascade whose goal is clot formation [61]. Heparin is a relevant glycosaminoglycan released in anaphylaxis that exhibits a key function in the activation of the contact system after degranulation of MCs and basophils. In addition, non–IgE-mediated immunologic anaphylactic reactions produced by the injection of heparin contaminated with oversulfated chondroitin sulfate have been described [123]. Given its negative charge, heparin is able to provoke the binding of high-molecular-weight kininogen (HK) to factor XII (FXII) of the contact system, inducing the autoactivation of FXII and the initiation of this cascade. Activated FXII induces the transformation of prekallikrein into kallikrein, a serine protease that hydrolyzes HK into bradykinin, one of the most potent vasoactive mediators involved during anaphylaxis [61,124-126].

The contact, coagulation, and fibrinolytic systems are intimately related because FXII activation in turn induces, via factor XI, the conversion of prothrombin to thrombin. This molecule is capable of transforming fibrinogen into insoluble fibrin, which, with the help of factor XIII, causes crosslinking of the fibrin polymers, thus creating clots [61].

A study of serum samples from patients with anaphylaxis mediated by insect stings showed that levels of factor V, factor VIII, and fibrinogen were decreased during the reaction [127]. On the other hand, regarding the contact system, other studies have shown decreased levels of FXII, prekallikrein, and HK, as well as elevated bradykinin values in sera from anaphylactic patients [128,129].

2.2 Cardiovascular System

The cardiovascular system is closely related to the pathophysiology of anaphylaxis [130,131]. A recent detailed review addresses the vascular disorders associated with this event, which is characterized by an acute increase in vascular

permeability, relaxation of vascular smooth muscle, and constriction of vessels in the thoracic cavity [27].

Cardiovascular disease increases the risk of severe anaphylaxis, and activated heart MCs release mediators in the thoracic cavity [130]. Kounis syndrome, a coronary hypersensitivity disorder caused by massive activation of cardiac MCs and characterized by acute myocardial damage, is associated with anaphylaxis [132,133]. Specifically, an increase in troponins has been described in patients with this syndrome and anaphylaxis [134-136]. Troponins are part of the contractile apparatus of skeletal and cardiac muscle cells and biomarkers of myocardial damage and myocardial infarction [137].

2.2.1 Cells and processes

Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) interact with each other and govern the vascular pathophysiological processes of anaphylactic reactions [27,115].

ECs form a monolayer called the endothelium, which functions as the physical barrier between blood and tissues, thus regulating vascular permeability and blood pressure [115,138-140]. In anaphylaxis, release of mediators during the reaction opens the adherens junctions, giving rise to an increase in vascular permeability and stimulating fluid extravasation [58,115].

VSMCs are responsible for the regulation of vascular tone through their contractile properties. This process is modulated mainly by changes in intracellular calcium levels and membrane depolarization [27,141,142]. In anaphylaxis, mediators act on these cells, initiating relaxing or contractile processes in bronchial and vascular smooth muscle cells [115].

2.2.2 Mediators derived from vessels

In anaphylaxis, ECs function as both receptors and effector cells releasing relevant agents such as nitric oxide (NO) [58,143-145]. This vasodilator and anti-inflammatory molecule is crucial in vascular homeostasis [146]. Specifically, mediators such as histamine and PAF induce synthesis of NO by activating endothelial NO synthase [52,58,143]. NO plays a key role in the pathophysiology of anaphylaxis by increasing the vasodilatation underlying the reaction, thus triggering intracellular calcium depletion in VSMCs. In addition, NO has a hypotensive effect and decreases the catecholamine levels [27,147]. Likewise, fractional exhaled NO is increased in patients with anaphylaxis who experience respiratory symptoms [148].

Endothelin-1 is a molecule with vasoconstrictor capacity, since it induces an increase in the amount of intracellular calcium upon binding to its receptors on VSMCs [149]. Murine models have shown that this mediator can induce MC degranulation [150]. In addition, levels of endothelin-1 have been shown to be elevated in the trachea of guinea pigs with anaphylaxis [151].

Vascular endothelial growth factor (VEGF) is a molecule released by cells such as ECs, VSMCs, and MCs that induces vascular permeability [58,152]. Levels of this molecule have been found to be elevated in the serum of a patient with anaphylactic shock [153].

2.3 Nervous System

The nervous system comprises the central nervous system, which includes the brain and spinal cord, and the peripheral nervous system, consisting of afferent sensory and efferent motor nerves [154]. This system monitors and responds to changes in the internal and external environment [155]. Therefore, it is essential for maintaining body homeostasis and reacting to acute stressors [156,157].

Numerous studies have described the importance of the nervous system in the induction and coordination of the immune response [156,157]. In allergic processes, the nervous system participates in activities such as regulation of antigen presentation, IgE production, and MC degranulation [156]. These functions are due to close associations between autonomic nerves and immune cells, which can be activated through numerous neurotransmitters, and molecules released through their activation can also influence the neuronal activity [156].

In anaphylaxis, histamine acts through C- and A-γ fibers, stimulating the release of substance P and calcitonin gene–related peptide from the peripheral nerve endings [158]. These neuropeptides induce further activation of MCs through their specific surface receptors and intensify the inflammatory response, thus affecting arteriolar vasodilation and increasing blood flow [156]. In addition, tryptase can activate protease-activated receptor 2 on sensory neurons, thus stimulating the release of substance P and calcitonin gene–related peptide [158,159].

Furthermore, IgE- and antigen-mediated MC activation induces the release of serotonin [160], which contributes to clinical signs of anaphylaxis and is associated with the severity of the episode [161]. In mice models, serotonin is released in response to ovalbumin and acts on the parasympathetic neuronal fibers. This event leads to release of acetylcholine, which acts directly by inducing bronchoconstriction [162]. Moreover, activated human and mouse platelets contain high concentrations of serotonin and were able to induce significant hypothermia, probably through 5-hydroxytryptamine receptor 7 (5-HT7) [160,163].

Adenosine is a significant molecule that is released by the nervous system during anaphylaxis [164]. Adenosine produces vasodilatation, increases vascular permeability, and potentiates mediator release from stimulated MCs [164]. Additionally, adenosine may act on the nervous system, causing the release of tachykinins from sensory nerve endings in a similar manner to bradykinin [165].

Finally, clinical studies highlight the role of the nervous system in recovery from anaphylaxis [166,167]. Epinephrine, norepinephrine, and angiotensin II are elevated minutes after onset of an anaphylactic episode [167]. The vasopressor activity of these mediators enables them to compensate for the vasodilation and extravasation of liquids produced during the reaction [34].

2.4 Other Circulating Blood Mediators

Blood-derived circulating biomarkers have several advantages, as they reflect the individual's status, are easy to measure, and are obtained using minimally invasive

methods [168]. Consequently, together with complement-, contact-, and coagulation-derived proteins, various circulating anaphylaxis-related molecules have been characterized and proposed as new molecular markers for this pathological event.

2.4.1 Circulating proteins

The blood contains many circulating proteins, among which human serum albumin stands out, since alone, it accounts for 55% of the total concentration [169]. A recent study of 112 patients with anaphylaxis showed that both serum protein concentration and human serum albumin levels decreased according to the severity of the reaction. Moreover, these measurements made it possible to indirectly quantify the extravasation underlying the event [25].

Oher circulating proteins have also been characterized in anaphylaxis. Apolipoprotein B (ApoB) was shown to inversely correlate with severity in a pediatric population of food-induced anaphylaxis [170]. Moreover, the decrease in serum apolipoprotein AI has been suggested as a promising biomarker for this pathological event [88]. In addition, a drop in this protein and apolipoprotein E levels has also been reported in the serum of anaphylactic mice models and in patients. However, apolipoprotein E has been shown to decrease in other inflammatory reactions; therefore, it could not identify anaphylaxis correctly [171]. Similarly, hemoglobin β subunit was found to be increased in 150 patients with mild hypersensitivity reactions and anaphylaxis [25].

2.4.2 MicroRNAs

microRNAs (miRNAs) are small noncoding RNA molecules (19-25 nucleotides) that govern the translation of messenger RNAs (mRNAs). Indeed, a single miRNA can modulate several mRNAs, and the same mRNA can be regulated by different miRNAs [172]. Thus, miRNAs participate in a wide range of physiological processes, such as proliferation, differentiation, and survival [173].

Alterations in circulating miRNA levels have been associated with a wide variety of diseases [174]. Furthermore, they may be associated with the individual's health status, making them noninvasive and promising biomarkers, although they are not yet used in clinical practice [173,175].

Diverse studies carried out in mouse models suggested the involvement of miRNAs (miR-155, miR-154-5p, miR-26a, miR-26b, miR-122a-5p, miR-135-5p, and miR-182-5p) in the allergic inflammatory processes underlying anaphylaxis [176-180]. In human samples, miRNA levels were shown to vary during this pathological event. One study in a pediatric population with food-induced anaphylaxis showed increased circulating serum levels of miR-21-3p and miR-487b-3p [181]. Other authors propose miR-451a as the most relevant biomarker in adult blood samples [88].

2.4.3 Extracellular vesicles

Extracellular vesicles (EVs) are lipid structures classified mainly according to their size and biogenesis into 3 subtypes: exosomes, microvesicles, and apoptotic bodies [182]. However, while they differ in terms of size, morphology, and protein composition, it remains difficult to distinguish between them [183].

EVs can be released by many cells under the physiological conditions controlling homeostasis [184]. They participate in cell–cell communication by regulating the interaction between various systems [183]. However, EVs are also released under pathological conditions [183,185,186]. Therefore, they have been proposed as a promising source of biomarkers, as they reflect the physiological or pathological state of cells. Moreover, they are very stable structures and are easy and inexpensive to obtain, since they can be isolated from many body fluids, such as blood and saliva [182,187].

The role of EVs in anaphylaxis is practically unknown. The only available study demonstrated the existence of a differential proteomic profile in EVs obtained during the anaphylactic reaction and revealed an increase in Ficolin-2, CDC42, and alarmin S100A9 levels [188].

2.4.4 Metabolites

In the last few decades, metabolomics has become increasingly important as a tool for biomarker discovery in various fields, such as allergy [189,190]. In anaphylaxis, a study carried out in guinea pigs demonstrated metabolic changes in challenged animals compared to controls [191]. Furthermore, several metabolites are generated from relevant known mediators participating in anaphylaxis, such as histamine. The rapid breakdown of this molecule gives rise to 2 metabolites (N-methylhistamine and N-methylimidazole octane), which are detectable in urine up to 24 hours after the onset of the reaction. However, although this sampling is less invasive than blood collection, its sensitivity and specificity is much lower [53,56,57]. In addition, given its stability in both urine and serum from anaphylactic patients, 9α,11ß-PGF2 appears to be a promising lipid-derived biomarker of anaphylaxis [84,87,88]. Moreover, a recently published exploratory study pointed to the relevance of other altered metabolites in anaphylaxis. Depending on the triggers or severity of the reaction, the differential molecules observed include glucose, lipids, cortisol, betaine, and oleamide [192].

3. Microenvironments

The relative contribution of mediators, cells, and systems to the pathophysiology of anaphylaxis is unknown. Furthermore, the location of effector cells and the half-life and abundance of the molecules released (local or systemic distribution) are likely to mask the recognition of reliable biomarkers. Identification of the exact mechanisms activated in these reactions would improve the diagnosis of patients based on precision medicine. Therefore, clarifying the various microenvironments established in anaphylaxis (eg, skin, lung, intestine, heart, vascular system, nervous system) will considerably enhance clinical management and recognition of better molecular markers.

3.1 Mast Cell Heterogeneity

Undoubtably, IgE-mediated anaphylactic reactions are the best characterized. However, the heterogeneity of

MCs is detrimental to finding a "universal" biomarker of anaphylaxis, and differences between humans and mice, and even between mice strains, indicate marked variations in IgE abundance, MC activity, and features of the mediators released [193,194]. Specifically, the degranulation profile of MCs, far from being simple, is not unique and uniform. Different types of stimuli and triggers induce diverse patterns of exocytosis, releasing various types of mediators to the surroundings [31]. MCs induce distinct lipid, cytokine, and chemokine secretion profiles, as well as diverse degranulation dynamics, depending on FcεRI cross-linking or other G protein–coupled receptor activation [30,195]. Such is the case of gastrointestinal symptoms, where the main MC mediators involved are PAF and serotonin, rather than histamine [196,197]. Moreover, the wide variety of MC receptors activating specific cell signalling has reached considerable relevance and may even have an impact on the diversity of organs affected in anaphylaxis [198,199]. For instance, A3 is increased 3-fold in lung MCs compared to skin MCs [90,200]. Therefore, different anaphylactic microenvironments would be created depending on the nature of each reaction. Consequently, the release of specific mediators derived from specific activated cells would explain the differences in the symptoms characterizing this pathological event.

3.2 The Vascular Wall and Homeostatic Factors

The endothelium plays a key role in the development of the reaction owing to its direct contact with blood. At the onset of anaphylaxis, many mediators released by the effector cells induce a direct effect on ECs. Among them, molecules such as histamine, cathepsin G, and PAF can produce vascular dilation and increase endothelial permeability [34,52,53,58,73,201].

On the other hand, products derived from the activation of the different blood systems are also capable of exerting an effect on the vascular wall. This can occur indirectly, for example, via complement-derived anaphylatoxins by inducing degranulation of effector cells [202,203], or directly, for example, via bradykinin, a potent vasoactive agent capable of inducing vasodilation and increasing vascular permeability [44,124]. In addition, a multitude of mediators released by effector cells, including the most relevant molecules (sT, chymase, histamine, and PAF), appear to be involved in the regulation of the complement, coagulation, and contact systems. Specifically, proteases play an important role by amplifying inflammation and activating the molecular cascades of complement and coagulation. For instance, chymase has been related to the coagulation pathway [48,56,61]. Similarly, sT and heparin can activate complement through an independent loop of the system's own convertases, thus

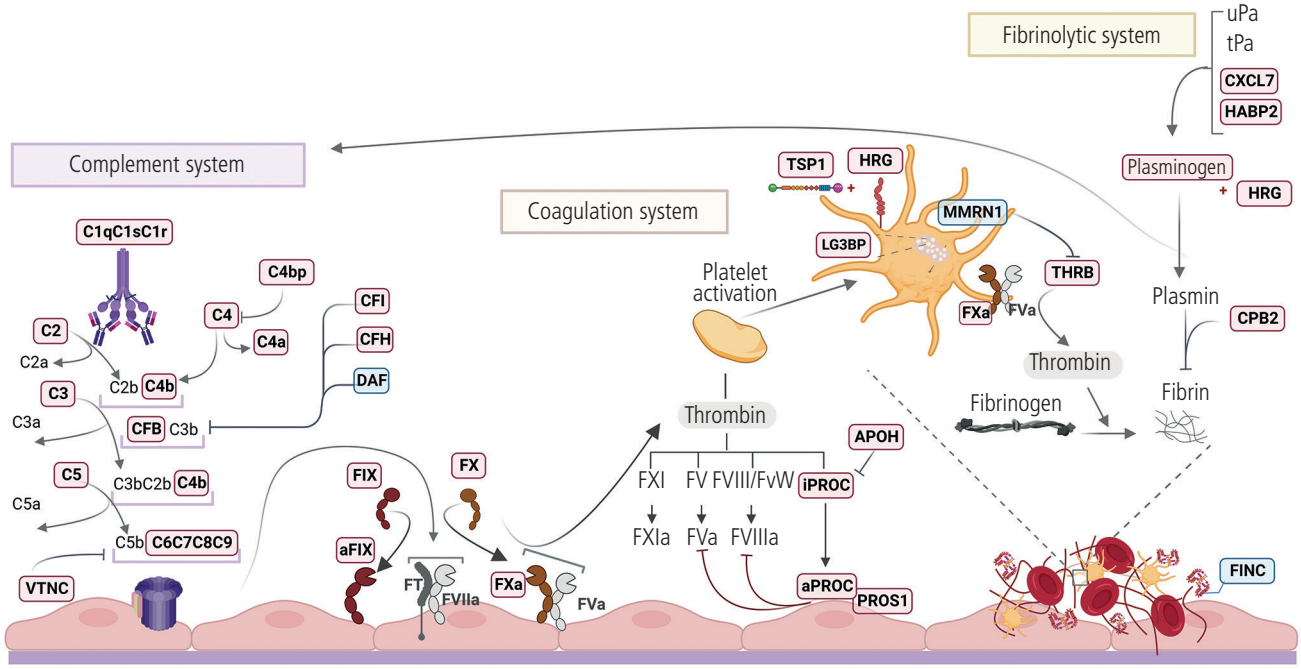

Figure 2. Relevant proteins identified in in vitro endothelial cells stimulated with serum from anaphylactic patients. Adapted from [115]. The complement, coagulation, and fibrinolytic systems stand out. Increased proteins are marked in red; decreased proteins are shown in blue. tPA indicates tissue plasminogen activator; uPA, urokinase; CXCL7, chemokine (C-X-C motif) ligand 7; HRG, histidine-rich glycoprotein; CPB2, carboxypeptidase B2; TSP1, thrombospondin-1; MMRN1, multimerin 1; THRB, thrombin; LG3BP, galectin-3-binding protein; FINC, fibronectin; APOH, apolipoprotein H; iPROC, inactive protein C; aPROC, active protein C; PROS1, protein S1; FV, VII, VIII, IX, X, XI, aXI, factor V, VII, VIII, IX, X, XI, active XI; C1qC1sC1r, complement components 1q, 1s, 1r complex; C2, 2a, 2b, 3, 3a, 3b, 4, 4a, 4b, 5, 5a, 5b, complement components 2, 2a, 2b, 3, 3a, 3b, 4, 4a, 4b, 5, 5a, 5b; C3bC2b, complement components 3b, 2b complex; C4bp, complement components 4b binding protein; C6cC7C8C9, complement components 6c, 7, 8, 9 complex; VTNC, vitronectin; CFI, complement factor I; CFH, complement factor H; DAF, complement decay-accelerating factor.

inducing the release of C3a and C5a [53,204]. In turn, the complement system can amplify coagulation and inhibit fibrinolysis. Reciprocally, the coagulation system has the capacity to activate the complement system [116].

An in vitro study of ECs stimulated with sera from anaphylactic patients highlights the relevance of platelets and coagulation processes in anaphylaxis and shows alterations in proteins related not only to this system, but also to the complement and fibrinolytic systems (Figure 2). Most of the increased proteins identified are related to the coagulation process, although it is noteworthy that, among them, the FIX and FX proteins had previously been observed to be decreased in patients' sera, indicating that the endothelium is actively participating in the consumption of factors derived from these systems during the course of the reaction and thus explaining the decrease in these factors in serum at the cellular level [115].

3.3 Amplification of the Molecular Loops of Interleukins

Inflammation in anaphylaxis is partially due to the contribution of interleukins to the reactions. These molecules not only mediate key immune processes, but also lead activation to the resident cells, setting up positive and negative feedback loops (Figure 3).

IL-4 is highly relevant in the development of T_H2 lymphocytes and the induction of IgE class-switching in B cells [205]. In turn, IL-13 activates IL-4 signalling pathways, induces IgE production, and activates recruitment of MCs and eosinophils, thus promoting their survival [205]. Both IL-4 and IL-13 exacerbate anaphylaxis through activation of a STAT 6–dependent pathway in murine models, increasing the sensitivity of target cells to vasoactive mediators [206]. In addition, both molecules could activate a phosphatidyl inositol-3 kinase (PI3K)–dependent pathway to induce expression of endothelial NO synthase and overproduction of NO [197]. Most of these processes lead to vascular permeability, vasodilation, and hypotension, thus influencing the severity of the episode. In addition, the fact that IL-4 and IL-13 shared the α chain of their IL-4 receptor (IL-4Rα) has led to the use of treatment with anti-IL-4Rα, which is considered a relevant therapeutic tool in anaphylaxis [207]. It has been shown that IL-4 and IL-13 can also enhance the response to other anaphylactic mediators in human MCs and macrophages by increasing the expression of their receptors, such as cysteinyl-leukotriene receptors [208].

IL-33 acts directly on IgE class-switching in B lymphocytes by inducing IL-4 and promoting MC degranulation [209]. Importantly, interleukins could amplify other signalling pathways; for example, IL-33 induces release of VEGF from MCs, increasing vascular permeability and contributing

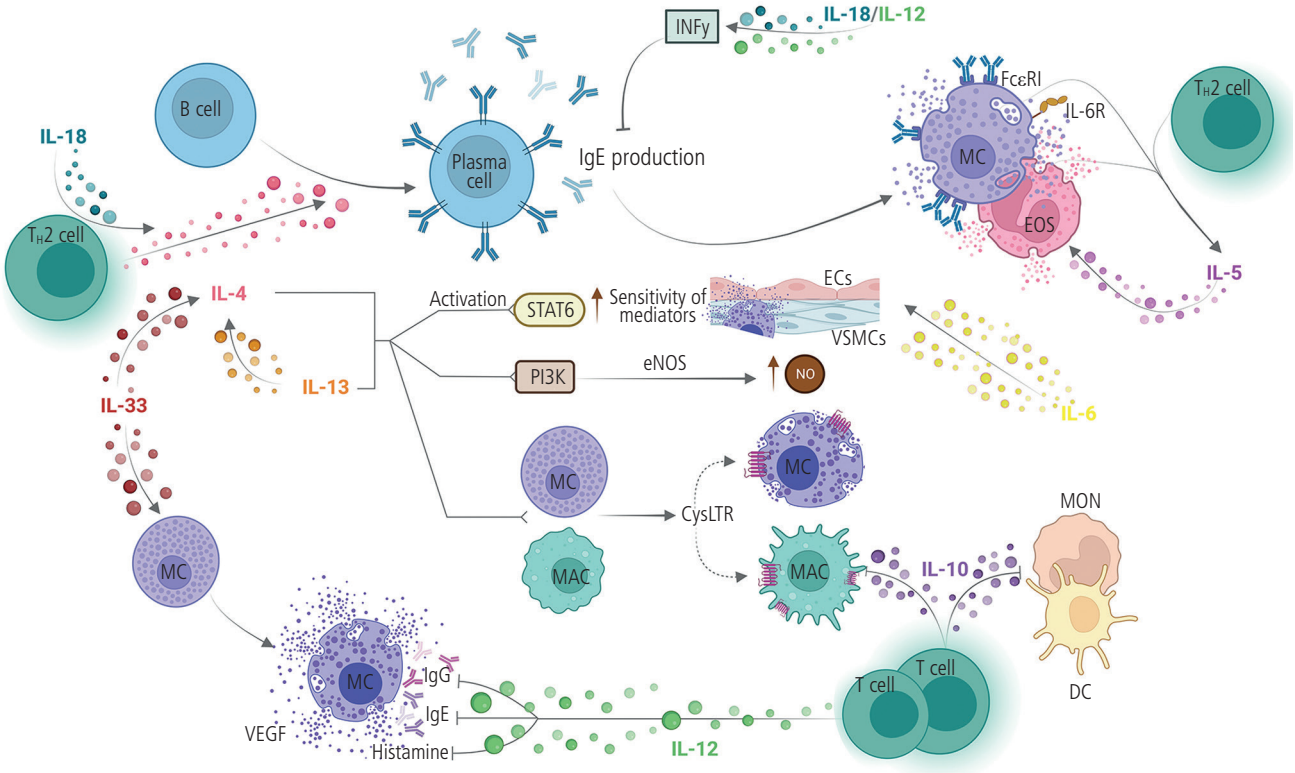

Figure 3. Relevant ILs exhibit positive and negative feedback loops in anaphylaxis. B cell indicates B lymphocytes; T_H2 cell, helper T type 2 lymphocytes; MC, mast cell; MAC, macrophage; EOS, eosinophil; MON, monocyte; MAC, macrophage; DC, dendritic cell; EC, endothelial cell; VSMC, vascular smooth muscle cell; IL, interleukin; VEGF, vascular endothelial growth factor; CysLTR, cysteinyl-leukotriene receptor; STAT 6, signal transducer and activator of transcription 6; PI3K; phosphatidylinositol 3 kinases; eNOS, endothelial nitric oxide (NO) synthetase.

to inflammation [210]. Likewise, the effect of IL-6 can be mediated by its binding to IL-6Rs present on the surface of various cells or in its soluble form (sIL-6R) [205]. IL-6 is also able to induce ECs to synthesize several factors and proteins of the complement system [211,212]. Finally, in vitro approaches show the effect of IL-3 in basophils by increasing expression of the activation markers CD69 and CD203c and enhancing mediator release in response to FcεR cross-linking [213,214].

In animal models, IL-18 has been shown to play a role in the initiation of T_H2 responses by inducing IL-4 and IL-13 synthesis in MCs and basophils, leading to an increase in IgE production [215,216]. Conversely, coadministration of IL-18 and IL-12 induces the production of IFN-γ, stimulates T_H1-mediated immune responses, and inhibits IgE synthesis [216,217]. Therefore, the administration of these molecules in combination could be considered a treatment for severe allergic disorders [217].

On the other hand, IL-10 modulates MHCII and expression of costimulatory molecules on monocytes/macrophages and dendritic cells, thus limiting the production of proinflammatory cytokines and chemokines [218-220]. Therefore, this mediator participates in the resolution of the systemic immune response. Specifically, peripheral T-cell tolerance in immunotherapy is mediated by upregulation of IL-10 by allergen-specific regulatory T cells [221]. In mouse models, the introduction of bioengineered microorganisms to deliver this protein to the intestine decreases food-induced anaphylaxis and prevents IgE-type sensitization to common food allergens [222]. IL-12 exhibits a similar function to that of IL-10. Its oral administration prevents and reverses peanut hypersensitivity. Furthermore, it was shown to reduce the release of histamine, specific IgE, and IgG1 in a mouse model of peanut anaphylaxis [223].

3.4 Communication Between Microenvironments: miRNAs and EVs

The data provided above demonstrate the importance of the different microenvironments in anaphylaxis. However, these are not isolated niches, since there is communication between them. Therefore, several molecules have been postulated as participants in the interaction between these microenvironments, including miRNAs and EVs, which have been described as key players in cell–cell communication during the anaphylactic reaction [181,188] (Figure 4).

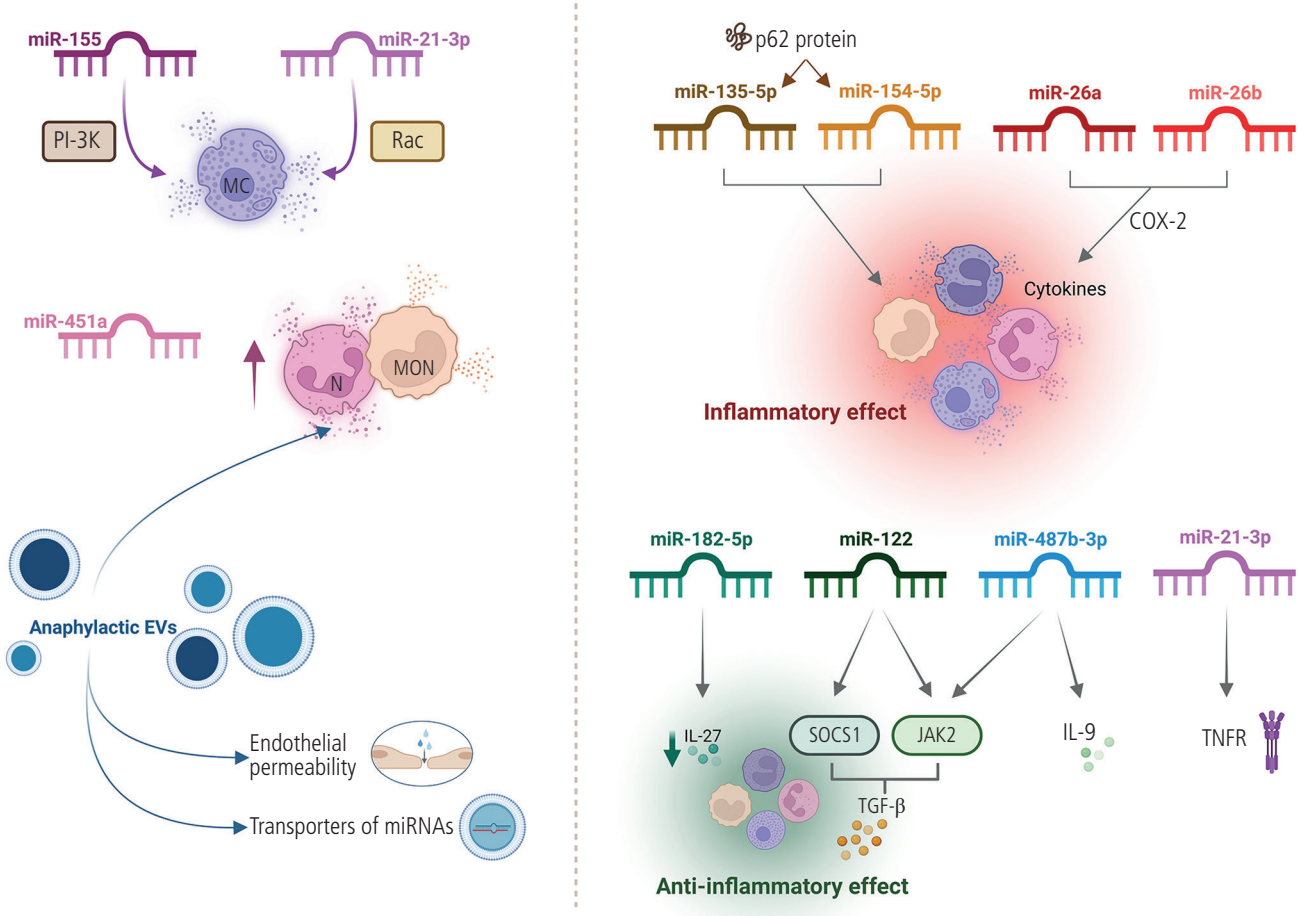

Figure 4. miRNAs and extracellular vesicles (EVs) described in anaphylaxis. These molecules are involved in degranulation and vascular permeability (left) and in pro/anti-inflammatory effects (right). MC indicates mast cell; MON, monocyte; N, neutrophil; PI3K, phosphatidylinositol 3-kinases; COX-2, cyclooxygenase 2; SOCS1; suppressor of cytokine signaling 1; JAK2; Janus kinase 2; TNFR, tumor necrosis factor receptor; TGF-β, transforming growth factor β.

miR-155 regulates degranulation of MCs by modulating their calcium concentration through PI3K levels [179]. In addition, miR-21-3p could participate in this process by its action on signaling of Rac, a protein involved in MC degranulation [181,224,225]. In turn, analysis of EVs obtained during anaphylaxis revealed that the main function of their differential proteins was neutrophil degranulation [188]. Specifically, elevation of circulating levels of miR-451a has been associated with increased degranulation of the effector cells in the reaction [88].

Nevertheless, EVs obtained during anaphylaxis have also been reported to induce an increase in endothelial permeability in vitro [188]. In addition, they can act as transporters of various miRNAs, which exert their action on the target cell [226]. Two mi-RNAs have been characterized in anaphylaxis, namely, miR-135-5p and miR-154-5p, which can regulate allergic inflammation in a p62 protein–dependent manner [179,227].

Other miRNAs play a role in the regulation of inflammation during anaphylaxis. For instance, miR-26a and miR-26b regulate levels of COX-2, a key enzyme in this process and in the release of the cytokines involved in the reaction [177]. Conversely, miR-182-5p has been reported to induce an anti-inflammatory effect, as it reduces the levels of IL-27, a cytokine involved in allergic inflammation [180]. Similarly, miR-122 was also found to participate in the inhibition of this process through the regulation of suppressor of cytokine signaling 1 and Janus kinase 2, both of which control the production of the anti-inflammatory mediator TGF-β [178]. In turn, miR-487b-3p modulates the inflammation underlying the reaction, as it participates in signaling of Janus kinase 2 and IL-9, a proinflammatory cytokine released by MCs [181]. Likewise, miR-21-3p regulates this process through its role in TNFR signaling, on which a wide variety of cytokines act [181].

4. Conclusions

Various molecules have been identified and proposed as biomarkers in anaphylaxis, although none of them has yet been extrapolated to clinical practice, probably because of the lack of studies connecting the clinical manifestations of the patient with the molecular mechanisms underlying the reaction. Accordingly, the recent increase in the understanding of the signaling pathways involved in anaphylaxis has led to new candidate molecular markers. Therefore, a more robust knowledge of the plethora of anaphylactic phenotypes and endotypes would lead to the identification and characterization of better diagnostic and predictive biomarkers that would in turn improve the clinical management of patients and their quality of life.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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