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ABSTRACT

Drug-induced anaphylaxis is a hyperacute reaction affecting multiple organs that can be of fatal consequence. Its incidence is increasing, consistent with a global increased sensitization to various allergens and drugs in the population. Few risk factors and mechanisms have been identified from human studies due to the rarity of anaphylactic events and their unpredictability. This systemic reaction is caused by the rapid release of a large range of functionally diverse mediators, including histamine and platelet-activating factor as the main drivers identified. Mechanisms defined from models of experimental anaphylaxis identify drug-specific antibodies of the IgE and IgG class that link the drug to antibody receptors on multiple cell types, causing their activation and mediator release. In the case of drugs with peculiar chemical structures, antibodies may not be necessary as drug-binding receptors, like mas-related G-protein coupled receptor member X2, have been identified. This review describes the complex reaction leading to drug-induced anaphylaxis that can involve various antibody classes, various cell types - including mast cells, neutrophils, platelets, basophils, macrophages and monocytes -, their mediators and receptors that, importantly, can be implemented alone or in association to participate in the severity of the reaction.

KEY WORDS

Anaphylaxis; drugs; IgE; IgG; MRGPRX2; platelet activating factor; histamine; serotonin; mast cells; basophils; neutrophils; platelets

ABBREVIATIONS USED

cPLA2: cytosolic phospholipase A2
CD32A: human activating IgG receptor FcγRIIA
FcεRI: high-affinity receptors for the Fc portion of IgE
FcγR: receptors for the Fc portion of IgG
$FcγR^{null}$ mice: mice deficient for FcγRI, FcγRIIB, FcγRIII and FcγRIV
$FcRγ^{-/-}$ mice: mice deficient for the FcRγ-chain, lacking all activating IgG and IgE receptors
FcRn: neonatal IgG recycling receptor
IL-4: interleukin-4
LuLISA: luciferase-linked immunosorbent assay
Mrgprb2: Mas-related G-protein coupled receptor member b2
MRGPRX2: Mas-related G-protein coupled receptor member X2
NETs: neutrophil extracellular traps
NMBA: neuromuscular blocking agents
NSAIDs: nonsteroidal anti-inflammatory drugs
PAF: platelet-activating factor
PAF-R: PAF-receptor
PAF-AH: PAF-acetyl hydrolase
PEG: polyethylene glycol

MAIN TEXT

INTRODUCTION

Anaphylaxis is a hyperacute reaction that can be of fatal consequence. It is a systemic reaction caused by the rapid and systemic release of a large range of functionally diverse mediators affecting multiple organs. These mediators typically induce urticaria, vasodilatation, increased vascular permeability and vascular leakage, edema and bronchoconstriction, leading to a drop in arterial pressure, tachycardia, bronchospasm and digestive troubles. Death can be caused by the resulting cardiac failure and/or asphyxia or pulmonary edema following major bronchospasm. Anaphylactic reactions cannot, in general, be foreseen. Due to their life-threatening nature, they represent an emergency situation for the medical staff.

The more recent publications describe a world incidence of anaphylaxis in humans between 50 and 112 episodes per 100 000 person-years, and drug allergy mortality is estimated at 0.05–0.51 per million people/year [1]. Interestingly, drug-induced anaphylaxis incidence is increasing, consistent with a global increased sensitization to various allergens in the population, including drugs [2]. Almost 60% of fatal anaphylaxis cases have been attributed to drugs [3, 4]. Due to their increasing availability, the anaphylaxis to mAbs jumped at an average rate of 0.77% of total anaphylaxis reports per year in the United States, from 2.00% in 1999 to 17.37% in 2019; it was the fastest increase observed among all the drugs responsible for anaphylaxis [5]. Surprisingly, very different drugs – whether considering chemical nature or structure, size, target, mode of action, biodistribution – lead to anaphylactic events with similar symptoms and consequences. The most frequent culprit drugs are antibiotics (mostly penicillin and cephalosporins), nonsteroidal anti-inflammatory drugs (NSAIDs), injected radiocontrast agents (iodinated contrast media and gadolinium), antineoplastic drugs, therapeutic antibodies and neuromuscular blocking agents (NMBA) used during surgery [3, 4, 6] Even more surprisingly, the size of most of these compounds are 100-1,000 times smaller than “classical” allergens - linked to allergic reactions to pollens, house dust mite, food allergens -, and due to this minimal size, these drugs would rather qualify as haptens (Figure 1): antibiotics, *e.g.* Penicillin 334 Da; Ciprofloxacin 331 Da; NSIADs, *e.g.* Ibuprofen 206 Da, Dicolfenac 296 Da; radiocontrast agents, *e.g.* Diatrizoate 613 Da; NMBA, *e.g.* Suxamethonium 361 Da, Rocuronium 530 Da. Their small size, allowing them to passively diffuse

systemically, could be interpreted as a common feature of drugs with anaphylactic potential. Nevertheless, drugs of radically larger sizes, proteins of 20-180 kDa including therapeutic antibodies, *e.g.* infliximab 149 kDa, cetuximab 152 kDa, or enzymes used for enzyme replacement therapy, *e.g.* glucocerebrosidase 60 kDa, and polymers 200-35,000 kDa contained in drug preparations like polyethylene glycol [7, 8] (Figure 1) that do not diffuse passively, are also reported to cause drug anaphylaxis with similar kinetics. Adding to the complexity, the route of administration of the drug responsible for anaphylaxis can be multiple: oral, infused, injected (intravenous, intradermal, subcutaneous, intramuscular). Concerning the inhalation route, some cases have been reported in asthmatic children using inhaled corticosteroids, probably related to milk protein traces [9]. This variability in chemical nature, size, biodistribution of culprit drugs for anaphylactic events makes it difficult to envision a single mechanism responsible for anaphylaxis induction.

Evidence of the mechanisms responsible for anaphylaxis from human studies is scarce due to the rarity of anaphylaxis and its unpredictability, and thus of the very few prospective clinical studies performed so far. Similarities between local allergic reactions (e.g. skin rashes, edema) and low-grade systemic anaphylaxis has led to proposing mechanisms of allergic reactions as the basis of severe anaphylactic reactions also without solid evidence to support them. Thus, clinical research in anaphylaxis has mainly focused on accumulating evidence of an "allergic" mechanism, including presence of certain mediators (e.g. histamine), enzymes (e.g. tryptase) and antibodies (e.g. IgE) classically involved in local allergic reactions. If histamine, tryptase and allergen-specific IgE are rather biomarkers than actual triggers of the anaphylactic reaction, which may be induced by other mechanisms entirely, will be discussed herein. As an example, histamine is found at elevated levels during anaphylactic reactions and proposed as the main mediator of anaphylaxis, but antihistamines do not demonstrate efficacy on severe anaphylaxis symptoms. Below are summarized risk factors and evidence from human studies to propose that anaphylaxis is an integration of diverse mechanisms leading to systemic organ failure rather than, simply put, an extreme allergic reaction.

RISK FACTORS & EVIDENCE FROM HUMAN STUDIES

Few risk factors have been identified that increase the risk of developing a drug-induced anaphylactic event. Whereas sex remains a matter of debate with controversies on higher rates of drug-induced anaphylaxis in women [3, 10], old age has been linked to both an increased risk of severe reactions and a higher incidence [11] with pre-existing cardiovascular morbidity being an important co-factor [4]. Surprisingly, atopy and allergic status of the patients does not appear to be convincingly related to higher risk of drug-induced anaphylaxis [6], suggestive that different or additional mechanisms may be at play in 'systemic' anaphylaxis compared to more 'local' allergic reactions. Nevertheless, patients with mastocytosis, a disease characterized by the presence of high numbers of mast cells in various organs, have a high occurrence of anaphylaxis [12], suggesting of a role of mast cells – the crucial effector cell of allergic reactions and inflammation [13]– in anaphylaxis.

Mast cells are notorious for the ability to quickly release histamine, the major mediator recognized in hypersensitivity reactions. Although antihistamines have not proven efficacious to prevent or treat severe anaphylaxis, intravenous administration of histamine in volunteers has been shown to reproduce most signs and symptoms of anaphylaxis, including cutaneous flushing, headache, airway obstruction, and transient hemodynamic changes, mainly evidenced by systemic hypotension and tachycardia [14, 15]. Thus, histamine has the capacity to mediate the symptoms of anaphylaxis, but is clearly not the sole mediator involved. Vadas *et al* indeed reported in their landmark study in 2008 that platelet-activating factor (PAF) levels in serum were directly correlated, and the activity of its degrading enzyme, PAF acetylhydrolase, inversely correlated, with the severity of anaphylaxis [16]. Their follow-up work [17] reported that histamine, PAF and tryptase, the major enzyme of mast cell secretory granules already identified as a biomarker for anaphylaxis [18], were all detected in serum of patients that underwent anaphylaxis of low- (grade 1), mild- (grade 2) and severe (grade 3) severity. However, serum concentrations of PAF and tryptase, but not histamine, correlated with anaphylaxis severity [17]. PAF is an extremely lipid mediator that can activate of a variety of cells that express the PAF-receptor (PAF-R), including endothelium, smooth muscle, and myeloid cells including mast cells. Thus, PAF could directly elicit the circulatory and respiratory symptoms of anaphylaxis while also eliciting the generation of other mediators involved in anaphylaxis propagation and severity.Intracutaneous injection or inhalation of PAF elicit symptoms resembling grade 1 anaphylaxis and bronchoconstriction ,

171 respectively, in human subjects [19, 20]. Even though deficiencies in the enzyme degrading PAF, PAF-
172 acetylhydrolase (*i.e.* leading to high levels of PAF) have been correlated with respiratory deficiencies in
173 asthmatic children [21], no study has linked it yet to anaphylaxis. Nevertheless PAF-acetylhydrolase
174 activity inversely correlates with anaphylaxis severity, and can be used as its marker [16, 22]. In contrast to
175 PAF, mast cell tryptase is not thought to elicit rapid responses that contribute to immediate manifestation
176 of anaphylaxis, although its effects are only partially described [23]. Mast cells in the vicinity of an
177 activated mast cell releasing tryptase have been reported to become activated in turn and to release
178 histamine [24]. Tryptase is considered mainly a 'practical' marker of mast cell activation as it can be easily
179 detected in serum [18]. Altogether, these data suggest that among identified immediate mediators with
180 potency to be anaphylaxis inducers, PAF, rather than histamine, is the contributor of the more severe
181 forms of anaphylaxis (grade 3) and potentially of lethality (grade 4) when highly abundant systemically.
182 More explorations of anaphylactogenic mediators (e.g. leukotrienes, prostaglandins) in human drug-
183 induced anaphylaxis remains to be performed to understand fully the mechanisms leading to moderate
184 and severe symptoms, or even to lethality.
185
186 Until recently, only one pathway had been universally accepted as the mechanistic explanation
187 of anaphylaxis induction: the IgE antibody pathway. Antibodies of the IgE class are generated at in
188 small quantities by B lymphocytes. Once produced, IgE antibodies have a very short half-life in
189 circulation as they cannot be recycled by the IgG recycling receptor FcRn. Total IgE levels are thus only
190 50-200 ng/mL in healthy individuals but generally several fold higher in allergics, with patients having
191 allergen-specific IgE levels up to 200ng/mL, particularly those that experienced anaphylactic events [25].
192 Direct evidence of the role of IgE in human anaphylaxis is based on transfer of purified human IgE in
193 the skin of human volunteers that transferred allergen reactivity [26] and several clinical trials using the
194 anti-IgE therapeutic antibody omalizumab: one suggesting less spontaneous episodes of anaphylaxis in
195 patients with mastocytosis [27], others proposing anti-IgE therapy as an adjunct therapy for allergic
196 desensitization, leading to fewer anaphylactic episodes [28-30].
197 Although the precise conditions for human B cells to start producing an IgE remain speculative
198 and extrapolated from data obtained in animal models [31], recent human studies found evidence that

allergen-specific IgE B cells arise from mature B cells producing initially an allergen-specific IgG [32]. Although elusive, human circulating non-secreting IgE B cells, *i.e.* IgE memory B cells, as well as non-circulating IgE-secreting B cells, *i.e.* IgE plasma cells, have recently been identified (in extremely low numbers) in the blood and bone marrow, respectively, of allergic patients [33, 34]. Indirectly supporting a role for these IgE-secreting cells located in the bone marrow in human anaphylaxis, bone marrow transplantation from allergic donors to non-allergic recipients has been reported in a few cases to transfer drug hypersensitivity, penicillin hypersensitivity for example (reviewed in [35]). Although several donor cell types in the allograft may contribute to the transfer of hypersensitivity, specific penicillin IgE could be detected 3 months after transplant, supporting the likely importance of graft-associated IgE-producing B cells[36]. This hypothesis is further supported by the observation that transplantation of livers from fatal anaphylaxis cases transferred food hypersensitivity (nuts or peanuts) to recipients with either detectable specific IgE or positive skin prick test (reviewed in [35]). Of note, IgE-producing B cells related to food allergy have been identified in the gut, and arise most probably from mature B cells producing initially a food allergen-specific IgA [37]. Wherever IgE is anatomically secreted - bone marrow, liver, gut - it has the unique ability to 'sensitize' human mast cells in tissue but also human basophils in blood, empowering them with the ability to react to a variety of specific targets, including allergens and drugs. This phenomenon, unique among antibody classes, relies on the IgE receptor FcεRI that these cells express constitutively. FcεRI is of such high-affinity that once bound an IgE remains on a mast cell for weeks [38]. Upon penetration of a drug/allergen in the body, it will bind to IgE-sensitized cells, provoke FcεRI aggregation on their surface, leading to cell activation, degranulation and mediator release, including histamine, tryptase and PAF.

Although IgE may be responsible for many cases of anaphylaxis to drug or allergen, it may be undetectable in others[39]. In such cases, the terms "anaphylactoid reactions" (i.e. anaphylaxis-like reaction) or "idiopathic anaphylaxis" (i.e. anaphylaxis of unclear trigger) may be applied. IgG antibodies are proposed as causative agents, and can be detected in some patients who react to NMBA [40], PEG [41], therapeutic antibodies and other drugs (reviewed in [42]). Although not proven, IgG antibodies could trigger activation of macrophages and other cells bearing Fcγ receptors, either directly inducting

anaphylaxis or acting in concert with IgE having the same specificity. Additionally, anaphylaxis-like reactions to certain drugs may be caused by direct interaction with Mas-related G-protein coupled receptor member X2 (MRGPRX2), [43, 44] which is expressed at high level in primary human skin and synovial mast cells, but not in primary lung mast cells [45]. Many drugs capable of directly inducing histamine release can bind and activate MRGPRX2 [46] (Figure 2).

MECHANISMS IDENTIFED IN EXPERIMENTAL ANAPHYLAXIS

Most animal models of systemic anaphylaxis are directly relevant to drug-induced anaphylaxis as they are based on the injection of a bolus of allergen/antigen/drug into a sensitized animal. Two main types of models are used. In passive systemic anaphylaxis, naive animals are directly injected with an anaphylactogenic mediator (e.g. histamine, PAF) or with antibodies thought to be responsible for anaphylaxis induction followed by a challenge with an allergen/antigen/drug in the next hours or days. In active systemic anaphylaxis, animals are exposed to low doses of an allergen/antigen/drug to induce an antibody response against that molecule followed by a challenge several weeks later. In the latter case, initial exposures can be performed in the presence or absence of an adjuvant. Surprisingly, models of active sensitization reveal that the presence or absence of adjuvant influences the principal mechanisms leading to anaphylaxis induction [47-49]. Thus, that each animal model of anaphylaxis, even each experimental protocol thereof, will draw a different picture of what pathways of anaphylaxis in humans may be (discussed in [42]). Even though data may be considered conflicting between studies, animal models have provided an enlightened understanding of the multiple mechanisms at play that are, in our view, the current basis of human anaphylaxis exploration and dogma: antibodies of the IgE and/or IgG class to the culprit drug triggering multiple cell types through activating antibody receptors, or mast cell activating receptors directly triggered by some drugs, notably Mas-related G-protein coupled receptor member b2 (Mrgprb2), the mouse ortholog of human MRGPRX2.

Passive systemic anaphylaxis. Injection of histamine or PAF in mice leads to symptoms resembling systemic anaphylaxis that are dependent on the presence of histamine receptors or PAF receptor (PAF-R), respectively [50]. Injection of allergen-specific IgE or allergen-specific IgG followed by allergen challenge (generally intravenous injection) hours to days later provokes systemic, sometimes

fatal, anaphylaxis that requires expression of FcεRI or IgG receptors (FcγR), respectively (reviewed in [47] and [48]). Both IgE and IgG receptors require cross-linking to trigger cell activation, implying that multiple IgE or IgG molecules need to bind the same drug molecule, or that the drug has been haptenized onto a carrier molecule to allow multimeric interactions. Among mouse IgG subclasses allergen-specific IgG2a and IgG2b are potent inducers, whereas IgG1 is weak [51], in line with its preferential binding to inhibitory mouse FcγR [52] and its rather anti-inflammatory role in mice [53]. Ciprofloxacin, an antibiotic of the fluoroquinolone family, can induce antibody independent anaphylaxis in mice. Mice lacking Mrgprb2 (the mouse orthologue of MRGPRX2) are protected from ciprofloxacin-induced anaphylaxis [44]. As is the case for MRGPRX2 in humans, Mrgprb2 in mice is expressed almost exclusively on mast cells and is since considered a direct target of several drugs belonging to fluoroquinolones, NMBA (*e.g.* atracurium, rocuronium) chemical classes and cationic peptides [43]. A novel mouse model allowing for the development of human mast cells expressing MRGPRX2 reported local mast cell degranulation after exposure to contrast agents, but did not investigate systemic reactions [54]. Altogether passive models of anaphylaxis validate antibody classes IgE and IgG and their receptors, Mrgprb2 and mediators histamine and PAF as potential inducers of anaphylaxis in simplified models (Figure 2), but are not able to rank them or discriminate among them for their relevance in human anaphylaxis.

Active systemic anaphylaxis. Mice sensitized with allergen in the presence of adjuvants show detectable IgE and IgG specific for the allergen, and develop anaphylaxis upon allergen challenge (intravenous, gavage) with severity increasing with higher doses of allergen. Surprisingly, IgE-deficient or FcεRI-deficient mice were protected from some active anaphylaxis models, but not others, demonstrating that the 'IgE pathway' is not necessary in some models active systemic anaphylaxis (reviewed in [48], [42] and [55]). In contrast, mice lacking all activating IgG and IgE receptors (FcRγ$^{-/-}$ mice) were resistant to anaphylaxis, as well as mice lacking only IgG receptors (FcγR^{null}) [56-58]. Transgenic expression in FcγR^{null} mice of a single [59] or of multiple human FcγR [57, 58] restored anaphylaxis, demonstrating the requirement of the 'IgG pathway' in severe active systemic anaphylaxis. Even though convincing animal studies on the potential contribution of the complement system to anaphylaxis are still lacking (discussed in [42]), some compounds trigger complement component C3a production leading

to myeloid cell activation through their complement receptors and, thus, to PAF and histamine release [60]. Mice deficient in either PAF-R or cytosolic phospholipase A2 (cPLA2) that is required for PAF generation (and, in the case of cPLA2, leukotriene and prostaglandin generation) had markedly reduced anaphylaxis symptoms [50, 56]. PAF-R antagonists consistently strongly inhibited anaphylaxis symptoms and protected from lethality in different mouse models of active anaphylaxis, whereas antihistamines had moderate to negligible effects, unless in concert with PAF-R antagonists [49, 51, 56, 61, 62]. Depending on the active systemic anaphylaxis model and/or the mouse strain used, tissue-resident mast cells and macrophages, and circulating basophils, monocytes and neutrophils have all been convincingly reported to be main (reviewed in [48], [42] and [55]). More recently platelets were added to this list through two independent reports using human activating IgG receptor FcγRIIA (CD32A) transgenic mice, proposing that platelets release pathogenic serotonin in response to FcγRIIA triggering on their surface by circulating IgG-antigen/allergen immune complexes (Figure 2) [58, 63] that form following exposure to high amounts of antigen/allergen as is mostly the case in drug anaphylaxis. The abundance and systemic distribution of platelets suggests a plausible role in anaphylaxis. Because humans and non-human primates, but not rodents, express FcγRIIA [52], the potential contribution of platelets in anaphylaxis models may be missed in mice lacking transgenic expression of FcγRIIA.

While animal studies suggest a diverse range of initiating pathways for anaphylaxis, demonstrating the contribution of each pathway in severe human anaphylaxis is challenging, since sampling of blood is typically undertaken after the reaction has occurred. Nevertheless, markers have been proposed to confirm the engagement of the IgE pathway (increase in interleukin-4 (IL-4) and soluble IL-4 receptor levels) and/or the IgG pathway (decrease in FcγR expression) [64], which have been reported to occur simultaneously in a mouse model of fatal anaphylaxis [61]. Our group demonstrated the reduced FcγR expression was a marker of the IgG pathway in passive and active mouse models of anaphylaxis [51, 57] and in our recent clinical study on NMBA-induced anaphylaxis (described in the next section) [40]. The diverse range of candidate effector cell types identified in animal studies makes their relative contribution difficult to comprehend in humans. The contributions from different cell types are

likely influenced by cell numbers, their capacities for activation, and the abundance of mediators generated per cell. Among circulating cells, platelets (150,000-450,000/μL) are ~70, ~700 and ~100,000-fold more abundant than neutrophils (2,500-6,000/μL), monocytes (200-600/μL) and basophils (1-3/μL). How these compare to mast cell and macrophage numbers is unclear as both cell types reside in various human tissues in which they differentiate into different subpopulations expressing different enzymes, receptors (including different IgG receptors and levels of MRGPRX2 for mast cells) and mediators [65, 66]. Skin mast cell densities in humans vary depending on the anatomical location [67]. Considering numbers only, platelets and neutrophils would probably largely dominate over mast cells and macrophages, whereas mast cells and basophils would likely be the dominant cell types activated through IgE and MRGPRX2-dependent pathways. Considering the high levels of specific IgG necessary for generating immune complexes with their target drug to trigger FcγRs, compared to few specific IgE-bound FcεRI on sensitized mast cells and basophils necessary to trigger their activation (Figure 2), IgE would largely dominate over IgG [68]. These considerations apply even more in anaphylaxis to ingested drugs/antigens that require the compound to reach circulation, as only as small fraction of the ingested compound is absorbed [69, 70]. Solving this equation is next to impossible, but informs clinicians of the possibility that one, two or even three different pathways, involving different antibody classes (or none), different receptors and different cells types in circulation and tissue resident, may be at play simultaneously in a drug-induced severe anaphylactic reaction. This next section will propose a clinician's view on drug-induced anaphylaxis, taking NMBA-hypersensitivity as an example.

NMBA-INDUCED ANAPHYLAXIS EXEMPLFLIES MULTIPLE MECHANISMS AT PLAY

As emphasized above with the animal models, the possible mechanisms leading to anaphylaxis in human begin to be better understood, as more and more actors are evidenced at the cellular or soluble levels that can interact and define complex endotypes. The example we chose to describe in this section in detail is NMBA-induced anaphylaxis during the perioperative period as it may be representative of drug-induced severe anaphylaxis implicating several pathways that synergize to increase severity.

The incidence of perioperative anaphylaxis varies between the geographical locations with rates of 1 in 10,000-20,000 anesthesia procedures. For the 2011-2012 period, in 714 patients who experienced perioperative anaphylaxis in France, the most common cause was NMBA administration (60%) [71]. The classical IgE-dependent mechanism that involves basophil and mast cell degranulation has been clearly documented in various studies for several years, mainly using the morphine quaternary ammonium (QA) as a surrogate epitope of antibody responses to NMBA, even if one can be critical on this laboratory reagent, the only one commercially available so far [72]. Interestingly, the historical hapten concept has been recently revisited by its author, Dr Werner J Pichler, who postulates now that in the minutes following the re-exposure to a drug, a massive mast cell degranulation occurs in response to IgE cross-linking by non-covalent drug-carrier complex called "fake antigen" [73]. However, concerning NMBAs, the absence of any sign of IgE-dependent immune activation despite evident clinical anaphylaxis in 10-20% of patients led us to test the hypothesis of an IgG-induced neutrophil activation, as we previously described in mouse models [56].

We prospectively conducted a multicenter study of 86 patients with suspected anaphylaxis to NMBAs during general anesthesia and 86 matched controls (age, sex, drug, type of surgery) [40]. We found that circulating anti-QA IgE was undetectable in a large percentage of the patients, whereas anti-QA IgG levels were significantly increased as compared to matched controls. Moreover, both anti-QA IgE and IgG levels correlated with anaphylaxis severity. We then found that down-regulation of FcγRs (CD32A, CD16) at the neutrophil surface was also associated with reaction severity, suggesting that anti-QA specific IgG formed immune complexes with NMBA to rapidly activate circulating neutrophils. This was further supported by increased neutrophil expression of CD11b and CD66b, elevated circulating levels of degranulated elastase and decreased PAF-acetyl hydrolase (PAF-AH) activity related to PAF secretion. Moreover, high levels of neutrophil extracellular traps (NETs), detected as DNA-myeloperoxidase complexes, were found in severe patients as compared to mild patients and to controls. Altogether, using a large panel of neutrophil activation markers, we could confirm that, in human, an IgG-dependent neutrophil activation occurs during NMBA anaphylaxis with, or independently of, IgE-dependent mast cell/basophil activation (Figure 3).

Supporting anti-NMBA IgG contribution to anaphylaxis and our finding that IgG receptor CD32A-expressing platelets can induce anaphylaxis in animal models, platelet activation in the same patient cohort suffering from NMBA anaphylaxis was associated with anaphylaxis severity and was accompanied by a reduction in circulating platelet numbers [58]. In order to better document IgG-mediated mechanisms in anaphylaxis, we isolated rocuronium-specific IgG from one patient and found that they could form immune complexes with rocuronium that could in turn activate neutrophils isolated from healthy controls, as evidenced by the activation of oxidative burst and NET release (Figure 3). These results reconcile clinical and experimental data on the role of IgE and IgG during anaphylaxis and can modify our biological diagnostic approaches to NMBA-induced anaphylaxis, even if skin tests remain major tools for IgE-mediated reactions. Indeed, we can now suggest to implement the classical biological evaluation of suspected NMBA anaphylaxis [74, 75] by exploring both IgE- basophil and IgG-neutrophil pathways.

Exploration of NMBA-induced anaphylaxis for the determination of specific IgEs against QA (as a surrogate epitope of NMBAs) and against each independent NMBA (rocuronium, atracurium, suxamethonium) can be made using commercial (ImmunoCAP, ThermoFisher) or home-made techniques, but the specificity is not optimal, false positivity and numerous cross-reactivities are observed [76]. The calculation of specific to total IgE ratio did not improve the biological diagnosis of rocuronium allergy [77]. Sensitization to NMBAs might originate from exposure to other drugs or compounds that contain also a QA epitope, like pholcodine (a morphine derivative contained in anti-cough medications): indeed, anti-pholcodine IgE can be detected in NMBA hypersensitive patients [78]. Altogether these recent studies emphasize the difficulties of correctly quantifying the circulating anti-NMBA IgEs. New methods are needed and we can assume that the recent luciferase-linked immunosorbent assay (Lu-LISA) that demonstrated 10-100-fold better sensitivity than ImmunoCAP for peanut allergen-specific IgE could be adapted to other allergens and drugs including NMBA-specific IgE detection, providing perhaps enhanced sensitivity and specificity [79]. In addition to anti-NMBA specific IgE, we now recommend, using similar techniques, to assay anti-NMBA specific IgG to increase the understanding of our clinical findings reported in 2019 [40].

Assays to detect soluble mediators can be used to invoke mechanisms involved in anaphylaxis. Histamine and tryptase measurements, routinely used to confirm an anaphylactic reaction, reflect the activation of mast cells and, in the case of histamine, basophils. An elevated level of serum tryptase remains one of the very best markers of anaphylaxis. However, the level of tryptase at baseline (after resolution of the anaphylactic reaction) is required to calculate the acute tryptase levels using the following algorithm: tryptase levels are acute if >[1.2×baseline tryptase] + 2 μg/L [80]. Baseline levels of tryptase are elevated in patients with mastocytosis (reflecting increased mast cell burden), and are associated with an increased risk of recurrent perioperative anaphylaxis [81]. As noted previously, human mast cells strongly express MRGPRX2 (which is also expressed more weakly by basophils in humans [82]). MRGPRX2 can directly bind a number of drugs leading to mast cell activation and mediator release such as tryptase [44], meaning that elevated circulating tryptase levels at the initial phase of anaphylaxis can thus been generated by IgE- or MRGPRX2-dependent mast cell activation, or both. Initially the list of drugs activating MRGPRX2 included NMBAs atracurium and rocuronium among others, but conflicting results have placed this assumption under debate [83, 84]. The ability and importance of rocuronium-induced MRGPRX2 activation is under evaluation, investigating effects of MRGPRX2 mutations [85, 86].

Both blood basophils and neutrophils can be studied ex vivo in the patients in order to improve diagnosis. The basophil activation test (BAT) is a useful tool to document NMBA anaphylaxis [87] that needs to be performed 4-6 weeks after the episode. This flow cytometry-based *ex vivo* assay can be adapted to other NMBAs [88]. The versatility of BAT testing may make it an increasingly utilized tool in the diagnosis of NMBA-induced anaphylaxis. Additionally, elastase levels (using ELISA) and DNA-MPO levels (markers of increased netosis) may be useful for detecting neutrophil activation during anaphylaxis (Figure 3) [89]. We can propose that a simple phenotypic study such as CD11b and CD66b expression, monitored over the course of a clinical reaction in parallel with tryptase, can document a potential specific anti-NMBA IgG-induced neutrophil activation at the time of the reaction in case of the presence of specific IgG.

CONCLUSION AND THERAPEUTIC AVENUES

Drug-induced anaphylaxis is: 1) a very severe clinical reaction that needs to be rapidly and extensively documented and diagnosed by adequate biological tools, 2) a complex reaction that can involve various cell types, mediators, receptors and intracellular pathways that can be activated alone or in association (Figure 2); NMBA-induced anaphylaxis being an ideal example.

Recent human clinical data proposed even further possible mechanisms in addition to MRGPRX2, exemplified by the role of contact system via factor XII activation and bradykinin release in some penicillin-induced anaphylaxis [90] , or after heparin injection and severe hypotension due to oversulfated chondroitin sulfate contamination [91]. Explorations outside of basophil activation, *i.e.* neutrophil, platelet and monocyte activation, and of anti-drug IgE, *i.e.* presence of anti-drug IgG [40, 41, 92], should increase in clinical research to improve our understanding of anaphylaxis and define markers for its endotypes. Because many anaphylactic reactions to drugs happen at first exposure, identifying potential cross-reactivities is of major importance to discourage use of some drugs in potentially susceptible patients; hypersensitivity to the oligosaccharide alpha-gal as a consequence of tick bites leading to cetuximab anaphylaxis is a good example [93]. In contrast, sensitization to some cereal and peach allergens (Lipid Transfer Protein) are high risk factors to NSAIDs anaphylaxis, without any cross-reactivity identified [94].

The first line treatment of any type of anaphylaxis, whatever the mechanism, is adrenaline (epinephrine). As far as therapeutic tools are concerned, the avoidance of the drug is the only efficient action when possible. If not possible, anaphylaxis might be prevented by pre-treating patients with the anti-IgE antibody omalizumab as it is known to be useful in drug desensitization [95]. Anti-drug therapy to prevent IgE engagement might also be considered: allergen desensitization by anti-cat allergen antibody therapy has indeed been reported already [96], and might be transposed to drugs [97]. Anti-drug therapy to capture the drug remains a poorly explored avenue to remove quickly the culprit drug and thereby arrest the ongoing anaphylactic reaction: attempts have been described in NMBA-induced anaphylaxis [98] due to the existence of a rocuronium and vecuronium capture reagent, sugammadex, but remains debated [99-101]. Unfortunately, a significant number of (IgE-mediated) sugammadex-induced anaphylactic reactions have been described [102-104], making this particular therapeutic compound non ideal to explore drug capture as a therapy for drug-induced anaphylaxis. Novel anti-drug therapies need

444 to be developed to understand the potential of drug capture to reduce anaphylaxis severity or even to
445 stop an ongoing anaphylactic reaction.
446

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FIGURES

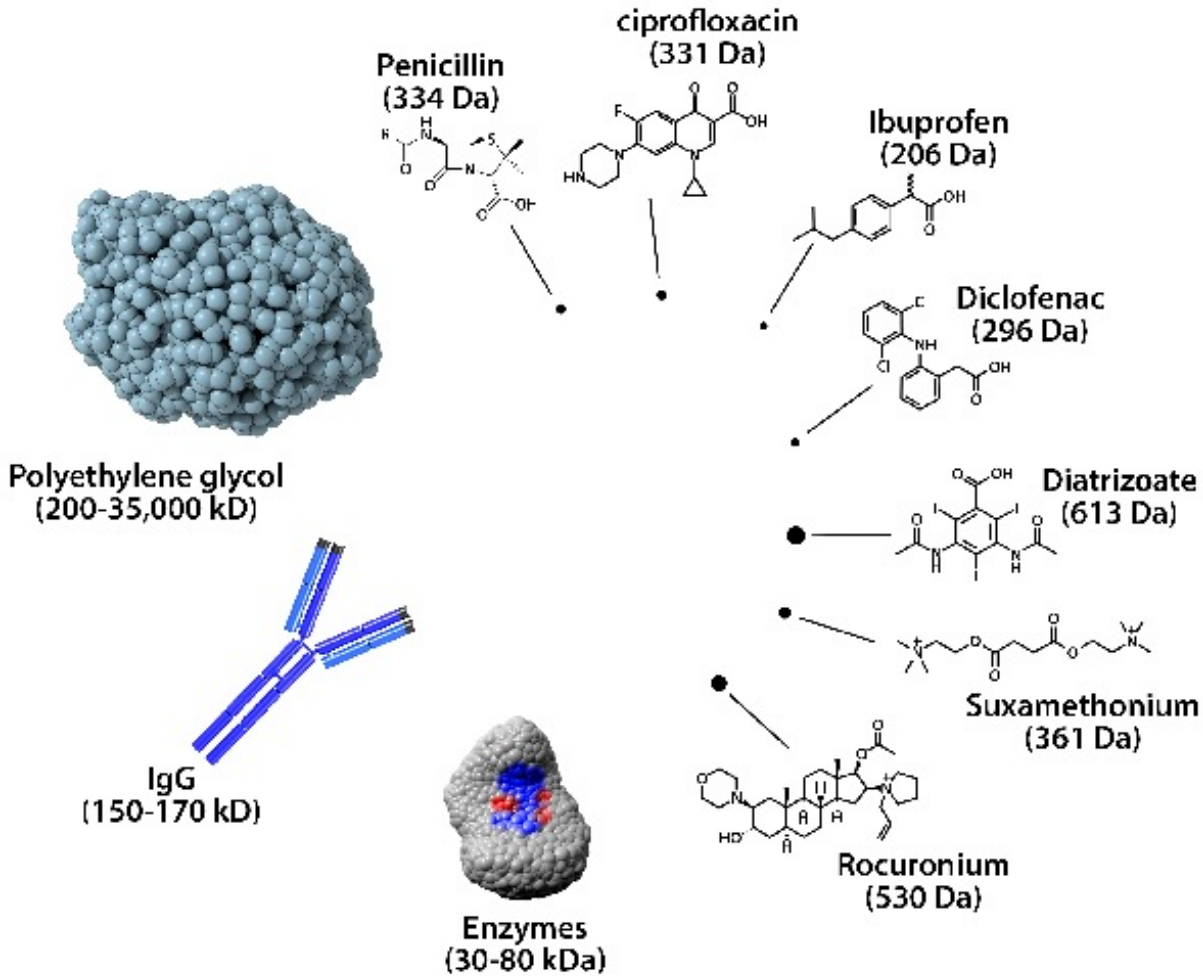

Figure 1. Relative drug sizes implicated in drug-induced anaphylaxis: size does not matter. Schematic representations of poly ethylene glycol (Image Credit: StudioMolekuul/Shutterstock.com), IgG, enzyme (Image credit; PDB 9LYZ), and chemical structures of indicated drugs and contrast agents. Dots represent the relative size of the depicted small molecules (<1 kDa) compared to those of PEG, IgG and enzymes.

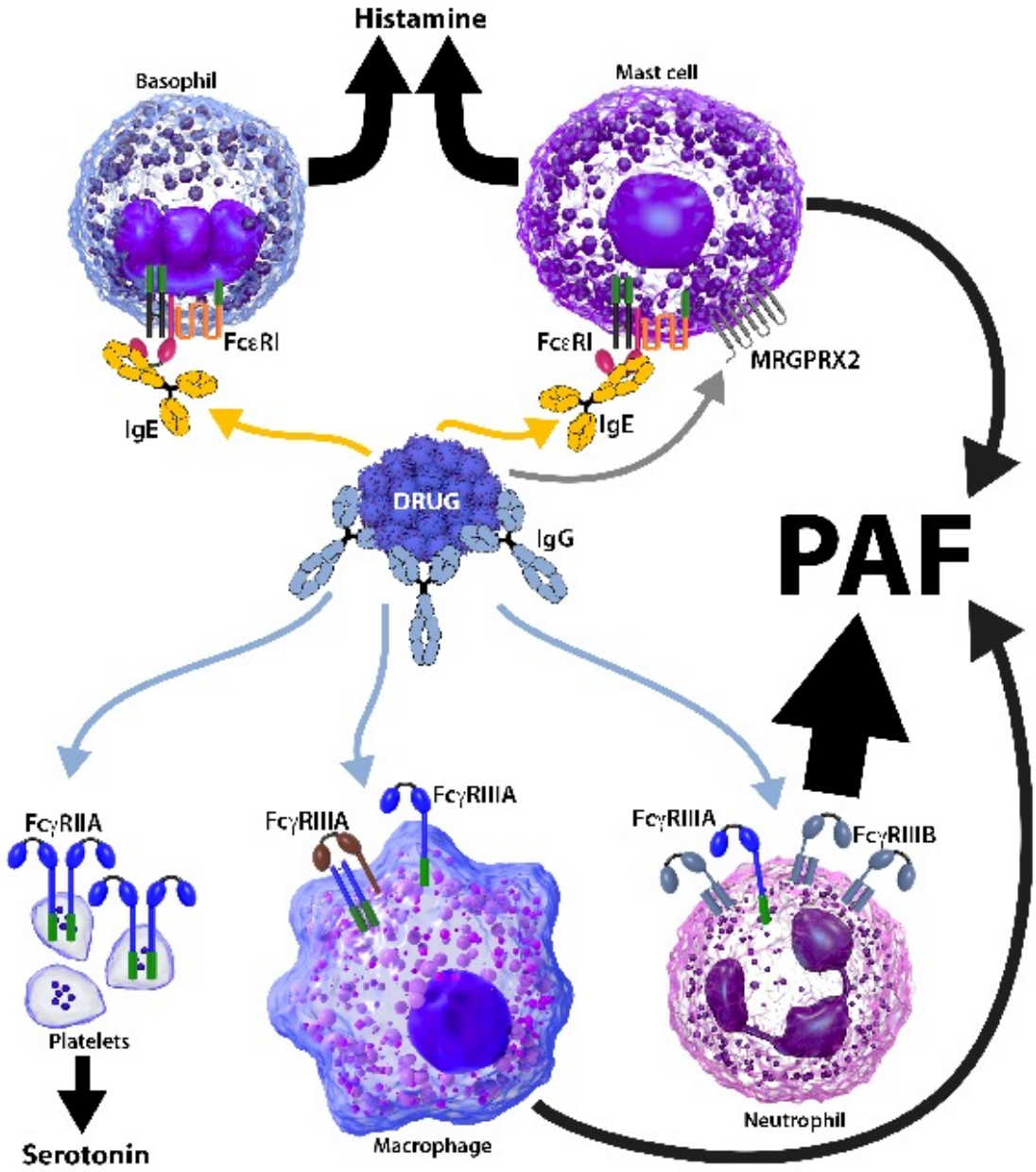

Figure 2. Potential pathways in drug-induced anaphylaxis. Once administrated a drug can be bound by (a) drug-specific IgE antibodies, pre-bound on their high-affinity IgE receptor (FcεRI)-expressing mast cells and basophils, leading to their release of anaphylactogenic mediators, histamine and to some extent PAF (*Note*: human mast cells are thought to make little or no serotonin); (b) drug-specific IgG antibodies, forming drug-IgG immune complexes that can bind to their low-affinity IgG receptor (FcγR)-expressing neutrophils (e.g. FcγRIIA and FcγRIIIB) and monocyte/macrophages (e.g. FcγRIIA and FcγRIIIA), leading to their release of PAF, and to FcγRIIA-platelets leading to their release of serotonin; (c) mast cell-expressed MRGPRX2 if that drug has affinity for this receptor, leading to mast cell degranulation and histamine and PAF release. The thickness of the green-colored arrows represent their contribution to the indicated mediator release.

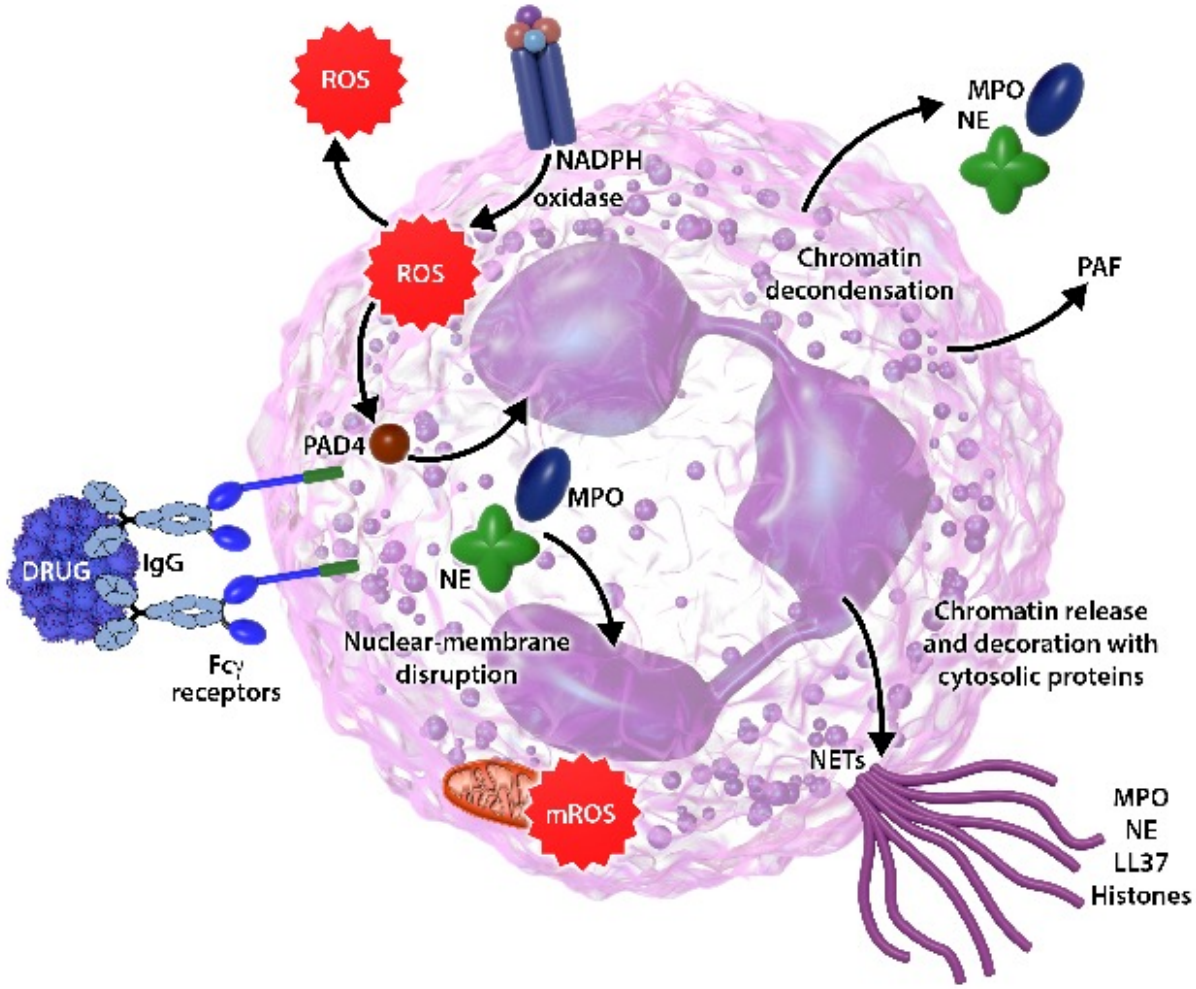

Figure 3. Mechanisms of IgG-induced neutrophil activation during drug anaphylaxis. The classical and historical pathway of anaphylaxis is based on mediator release by mast cells and basophils activated by the engagement of FcεRI after their interaction with a drug/anti-drug IgE immune complex (IC). A second pathway was recently demonstrated both in mice and human. The drug can react with specific IgG and form an IC that binds to several FcγRs at the neutrophil surface and activate the cell. In addition to reactive oxygen species (ROS) and proteases release such as neutrophil elastase (NE) and myeloperoxidase (MPO), neutrophils release platelet activating factor (PAF) and neutrophil extracellular traps (NETs), also involved in anaphylaxis clinical manifestations. The release of NETs is the consequence of ROS production, in particular due to mitochondrial-derived ROS (mROS) production and peptidyl arginase deiminase 4 (PAD4) activation leading to chromatin decondensation, nuclear membrane disruption and chromatin extracellular release.