About the role and underlying mechanisms of cofactors in anaphylaxis

F. Wölbing, J. Fischer, M. Köberle, S. Kaesler & T. Biedermann

Department of Dermatology, Eberhard-Karls-University of Tübingen, Tübingen, Germany

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Abstract
Anaphylaxis is the systemic and most severe presentation of type I allergy. A number of conditions were identified that modulate the onset of anaphylaxis such as co- or augmentation factors, which significantly lower the allergen dose necessary for triggering anaphylaxis. Next to physical exercise or alcohol consumption, co-administration of nonsteroidal anti-inflammatory drugs (NSAID) or concomitant infectious diseases are well-documented cofactors of anaphylaxis. Registries for anaphylaxis document a role for cofactors in about 30% of anaphylactic reactions. Some disease entities such as ‘wheat-dependent exercise-induced anaphylaxis’ (WDEIA) are explicitly characterized by elicitation of anaphylaxis only in the presence of at least one such cofactor. Using WDEIA as a model disease, studies demonstrated that exercise increases skin prick test reactivity to and bioavailability of the allergen. Additional data indicate that alcohol consumption and NSAID administration display similar effects. Modulation of the cellular activation threshold is another mechanism underlying cofactor-induced anaphylaxis, most likely also functional when infectious diseases orchestrate elicitation of anaphylaxis. Cofactors are increasingly accepted to play a fundamental role in eliciting anaphylaxis. Consequently, to improve patient management modalities, a better understanding of the underlying mechanisms is warranted. This review aims to update clinicians and clinical scientists on recent developments.

Classically, the allergen-induced cross-linking of IgE antibodies bound to high-affinity Fce receptors on mast cells initiates signal transduction and the release of preformed mediators like histamine, which elicit the clinical symptoms of type I allergic reactions. The most severe presentation of type I allergy is called ‘anaphylaxis’, defined as a generalized immediate-type hypersensitivity reaction. Anaphylaxis is clinically characterized by involvement of more than one organ system, in particular the skin, the gastrointestinal tract, the respiratory, and the cardiovascular system. The symptoms comprise relatively mild urticaria or diarrhea but also possibly life-threatening anaphylactic shock (1).

Available epidemiological data regarding anaphylaxis are widely varying, most likely due to incoherent definitions of anaphylaxis and a lack of reporting. Published studies estimate a lifetime prevalence between 0.05% and 2% and an incidence of 3.2–68.4 per 100 000 patient-years (2, 3). Although anaphylaxis seems to be triggered by sole allergen contact in most cases, the role of additional factors, also referred to as co- or augmentation factors, for the elicitation of anaphylaxis is increasingly accepted. For the first time, cofactor-dependent anaphylaxis was described in 1979 by Maulitz et al. (4) who described a patient with ‘exercise-induced anaphylaxis to shellfish’. Meanwhile, physical exercise is the best studied cofactor of anaphylaxis and ‘food-dependent exercise-induced anaphylaxis’ (FDEIA) is accepted as a defined clinical entity. Other well-documented cofactors of anaphylaxis are nonsteroidal anti-inflammatory drugs (NSAID) like acetylsalicylic acid (ASA), alcohol consumption, and infectious diseases in general (5–12). According to the anaphylaxis registries of different European countries, next to exercise, alcohol consumption is a relevant cofactor in up to 2.5% of anaphylactic events and drugs such as ASA were registered as a cofactor in 6.1–9% of severe anaphylactic reactions (Table 1). The role of infections as cofactors of anaphylaxis is reported to be relevant in 2.5–3% of anaphylactic reactions in children and in 1.3–11% in adults (8, 13–18). Infections are particularly dangerous for patients...
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with a risk to develop anaphylaxis, because in contrast to most other relevant cofactors of anaphylaxis, infections cannot simply be avoided or foreseen.

Extrapolation from different studies and registries suggests that cofactors play a role in about 30% of all anaphylactic reactions in adults (Table 1). These data highlight the importance of recognizing cofactors in patients with anaphylaxis and of including them into diagnostic measures. Detailed questions about a possible involvement of cofactors must be part of each allergologist’s patient routine assessment. Identifying both the eliciting allergen and the dependence on cofactors is pivotal for patients’ diagnostic measures, risk assessment, and doctors’ advice for the patients, which should help to avoid possibly life-threatening anaphylactic events in the future.

Thus, following the increasing awareness of the role of cofactors for anaphylaxis, there is a need for a better understanding. This should also help to develop more specific treatment strategies. The research in this field is just emerging, but initial data indicate two major levels of cofactor-induced modulation eliciting anaphylaxis: increased bioavailability of the allergens and decreased activation threshold on the cellular level (Table 2). Thus, allergen doses not sufficient to induce anaphylaxis in the absence of cofactors become dangerous triggers of anaphylaxis (as schematically shown in Fig. 1) in susceptible patients (19).

**Exercise as cofactor of anaphylaxis**

Exercise as a cofactor of anaphylaxis was first reported by Maulitz et al. They reported on a patient who developed anaphylactic symptoms two times following jogging after shellfish ingestion, whereas physical exercise as well as shellfish alone was tolerated (4). This constellation emerged to be no singular event and later on was defined as the separate disease entity of ‘food-dependent exercise-induced anaphylaxis’ (FDEIA). Although exercise does not exclusively trigger anaphylaxis in food allergic patients, FDEIA patients are the most relevant group of patients with exercise-triggered anaphylaxis (EIA). Meanwhile, numerous case reports describe FDEIA to pistachio, spinach, meat, shrimps, wheat, and many more (20, 21) and also for the more recently described entity of delayed type I allergy to red meat in an elderly lady that was sufficient to trigger anaphylaxis to a meat loaf (24).

To date, the best characterized EIA syndrome which therefore can serve as a model disease is FDEIA following consumption of products containing wheat (25). This most prevalent subform of FDEIA, termed ‘wheat-dependent exercise-induced anaphylaxis’ (WDEIA), was characterized in detail by Palosuo et al. (26). Later, it was demonstrated that in most WDEIA patients, conventional tests to verify sensitization to wheat remain without reaction (27), but IgE
directed against α-5-gliadin (Tri a 19), an ethanol-soluble wheat protein, is most valuable to diagnose WDEIA (28, 29). The current understanding of the pathophysiology of exercise-induced anaphylaxis to allergens focuses on two levels of cofactor modulation:
1 Exercise increases the bioavailability and influences the distribution of certain allergens, 
2 Exercise decreases the threshold for activation of mast cells and basophils.

Investigations on WDEIA showed that both, exercise and ASA, increased intestinal absorption of allergens possibly by establishing a leakage of the intestinal barrier (see also Fig. 2). Matsuo et al. (30) could show significantly increased uptake of gliadin in humans after consumption of wheat followed by adequate physical exertion. One possible underlying mechanism is intestinal barrier dysfunction; however, a high intensity and a long duration of more than 8 h were shown to be necessary to obtain a relevant barrier dysfunction (25). This indicates at least additional other mechanisms of action, because clinical observation shows that the intensity of exercise necessary to trigger FDEIA in humans varies quite tremendously from exercise near exhaustion to very mild. Interestingly, a mouse study on gastrointestinal lysozyme uptake showed that not only exercise but also lysozyme-specific sensitization significantly upregulated intestinal absorption and that sensitization and exercise together synergistically increased this uptake demonstrating the interdependence of IgE reactivity and exercise (31). In conclusion, available data indicate the following scenario: the intensity of exercise and the degree of sensitization coregulate the intestinal absorption of allergens with the consequence of orchestrating anaphylaxis each on an individual level.

Next to allergen bioavailability, exercise seems to influence the threshold for activation of mast cells and basophils. Regarding mast cells, this assumption is mainly based on data from immediate-type skin test reactions. These were more marked if patients underwent a defined form of exercise prior to testing (32, 33). One explanation for this decreased cellular activation threshold that is discussed is increased plasma osmolality, because intense exercise leads to an increased serum osmolality and changes in pH (25, 34, 35). However, in vitro studies confirming this hypothesis applied osmolality levels of at least 340 mOsm normally not reached under exercise or even under pathologic conditions, which only slightly exceed physiological values of 280–290 mOsm (25, 34, 35). Alternative concepts postulate that activation of tissue transglutaminase (tTG) in the intestinal mucosa is able to enhance degranulation on a cellular level and that exercise-induced release of endorphins may enhance MC or basophil activation. The first hypothesis is based on observations

| **Table 2** Proposed mode of action of the most important cofactors of anaphylaxis |
|-------------------------------------------------|-------------------------------------------------|
| Increased intestinal allergen absorption | Increased cellular activation |
| Exercise | Tight junction dysregulation (25, 30, 31) | Hypothetically due to increased plasma osmolality (25, 34, 35), activation of intestinal transglutaminase (36), or endorphin release (37, 38) |
| ASA | Tight junction dysregulation (30, 40, 41) | Idiosyncrasy due to cyclooxygenase blockade (42, 43) |
| Alcohol | Tight junction dysregulation (25, 54) | Cellular activation by innate immune receptors (68–71), anaphylatoxins (74, 75), or via FcγR (63) |

**Figure 1** ‘Threshold dose’ model of cofactor-dependent anaphylaxis. High allergen doses induce strong anaphylaxis, while low allergen doses induce subclinical allergic reaction but no anaphylaxis. In contrast, low ‘subthreshold’ allergen doses in combination with cofactors trigger strong anaphylaxis.

**Figure 2** Modulation of the intestinal allergen absorption. In the presence of cofactors like alcohol or exercise, the intestinal allergen absorption is increased. The more rapid allergen uptake results in higher peak concentrations allowing even low amounts of allergen to touch or exceed the threshold for inducing anaphylaxis.
that the cytokine interleukin (IL)-6, whose expression is 50- to 100-fold increased in marathon runners, upregulates tTG which by modifying especially α-5-gliadin proteins allows the formation of large peptide aggregates more effectively cross-linking the high-affinity IgE receptor FcεRI (36).

The latter hypothesis is based on in vitro experiments showing that beta-endorphin induces human mast cell degranulation and histamine release (37). Investigations in chronic allergic rhinitis patients confirmed that nasal pretreatment of sensitized patients with beta-endorphin significantly enhances histamine levels in the nasal fluid after allergen challenge, while nasal beta-endorphin treatment alone did not (38). However, such effects were not yet analyzed in anaphylaxis.

Consequences for the clinician

Even though scientific analyses are ongoing, at present the following can be concluded: (i) exercise increases the intestinal allergen absorption and (ii) the clinical relevance of this effect and the intensity of exercise necessary to trigger anaphylaxis in a patient depend on various individual factors such as the sensitization pattern. Therefore, a complete diagnostic assessment in patients with suspected FDEIA must always include provocation tests to identify the eliciting allergen/food and to assess the individual risk of anaphylaxis. The most important differential diagnoses of FDEIA are cholinergic urticaria, exercise-induced asthma, and physical urticaria. It should also be considered that in some patients, more than one cofactor is necessary to elicit anaphylaxis such as exercise and alcohol or exercise and ASA.

Drugs as cofactors of anaphylaxis

NSAID

Some groups of drugs can modulate the onset of anaphylactic reactions triggered by allergens independent of a drug-specific sensitization. In this respect, evidence is best for a role of NSAID as cofactors of anaphylaxis with the first report published in 1984 (39). Epidemiological data—even though relatively sparse—suggest that NSAID trigger anaphylaxis in 1.2–4.7% of all reported anaphylactic events (Table 1). Mechanisms underlying “idiosyncrasy” (nonimmunological hypersensitivity) to NSAID probably contribute to its role as cofactor of type I allergy, but underlying mechanisms are still incompletely understood. Experimental data, however, prove NSAID’s role in augmenting type I allergic reactions. Flemström et al. (40) investigated a model of dextran allergy in passively sensitized guinea pigs. While intragastric dextran administration alone caused no reaction, addition of ASA triggered anaphylaxis. These results suggest that similar to exercise, intestinal absorption of antigen can be upregulated by administration of ASA. Indeed, investigating the absorption of gliadin in humans with and without concomitant intake of ASA demonstrated that ASA increased the amount of serum gliadin 30 min after wheat consumption by fivefold (30). As one possible underlying mechanism, a dysregulation of tight junctions establishing the intestinal barrier in the gastrointestinal epithelium was postulated (30, 40). Indeed, treatment with 5 mM ASA decreased production of the tight junction protein claudin-7 and significantly increased dextran permeability in an in vitro model (41).

Of note, idiosyncratic reactions in response to NSAID such as urticaria or gastrointestinal symptoms are thought to be related to the nonselective blockade of cyclooxygenase 1 and cyclooxygenase 2 by NSAID. As compensation, synthesis of leukotriene A4 from arachidonic acid via the 5-lipoxygenase is increased. The activation of the LTC4 synthase then results in an enhanced release also of other leukotrienes derived from leukotriene A4. Susceptible patients display idiosyncrasy to NSAID, some of them based on a polymorphism in the promoter region of the leukotriene C4 synthase (42, 43). The pattern of symptoms in patients with idiosyncrasy such as generalized urticaria, angioedema, and dyspnea resembles that of anaphylactic reactions and idiosyncrasy of the gastrointestinal tract, even though less well known, is frequent. This indicates that the mechanisms of NSAID idiosyncrasy may also be underlying changes in the gastrointestinal barrier postulated in cofactor-induced anaphylaxis. In addition to these effects, in vitro pretreatment of mast cells with ASA directly modulated FcεRI-dependent mast cell degranulation and LTC4 release following FcεRI stimulation (44, 45). Moreover, several studies could show that also in humans, systemic administration of ASA triggers increased skin test reactions to different allergens (30, 32), supporting the concept that ASA next to modulating the intestinal absorption of allergens also directly modulates effector cell function in cofactor-induced anaphylaxis.

Other drugs relevant to anaphylaxis

In general, it can be assumed that drugs that are able to cause mediator release from mast cells and basophils are potential triggering factors of IgE-mediated anaphylactic reactions and thus may act as cofactors of anaphylaxis. Important examples of such drugs are X-ray contrast media (in general, iodinated contrast media, most frequently iomeprol and iopromide) (46), muscle relaxants (most frequently suxamethonium) (47), certain antibiotics like DNA gyrase inhibitors, and some opioids (48, 49). Another mechanism of triggering anaphylaxis may be supporting allergen persistence. H2-receptor antagonists and the so-called proton pump inhibitors (PPI) blocking or inducing long-lasting suppression of the gastric acids may lead to allergen persistence, especially of degradable allergens. Gastric acid digests and thereby modulates or inactivates protein allergens. Both drugs interfere with these functions by increasing the gastric pH. This results in less efficient inactivation of food allergens, and otherwise, labile allergens may reach the intestine and induce local or systemic allergic reactions. Indeed, studies in mice confirmed that the use of PPI increases the risk of sensitization to food allergens and the risk to develop anaphylaxis (50). Even in humans, it was shown that 25% of all patients tested developed clinically relevant IgE against food allergens while being on PPI (51). This is especially relevant...
for patients with oral allergy syndrome to acid-sensitive allergens that are at risk to develop systemic type I reaction following high allergen intake while being on PPI (51, 52). The inhibition of beta-adrenergic signals on effector cells of anaphylaxis, such as mast cells and basophilic granulocytes, by beta-adrenoceptor antagonists leads to inhibition of the cyclic AMP system and consequently to a destabilization of these cells (53). In addition, beta-adrenoceptor antagonists inhibit important blood pressure regulating mechanisms, both facilitating the induction and severity of anaphylaxis. However, the significance of these mechanisms for triggering anaphylaxis is still a matter of debate. A recent multicenter study failed to confirm that treatment with beta-adrenoceptor antagonists is a predictive parameter for the occurrence of severe anaphylactic reactions in a cohort of hymenoptera venom allergic patients (9).

Consequences for the clinician

Emerging evidence indicates that different drugs can act as cofactors of anaphylaxis. Especially NSAIDs are frequently identified as cofactors of anaphylaxis. Consequently, NSAIDs are to be included in diagnostic measures and patients should be informed about their relative risk. It should be especially recommended to avoid the combined intake of identified food allergens and NSAIDs. If muscle relaxants or X-ray contrast media are suspected to induce anaphylaxis or anaphylactoid reactions, they should either be avoided or premedication with corticosteroids and antihistamines should be recommended. In case of PPI medication, patients with oral allergy syndrome should be informed about the possible role of PPIs to act as cofactors of anaphylaxis through incomplete acid-dependent digestion. Beta-adrenoceptor antagonists are used in patients with myocardial infarction, cardiac arrhythmias, and severe heart failure, and the risk associated with avoiding beta-adrenoceptor antagonist treatment may outweigh the risk of anaphylaxis. The decision of avoiding beta-adrenoceptor antagonists has to be taken on an individual basis.

Alcohol as cofactor of anaphylaxis

Alcohol consumption was shown to facilitate the manifestation of food allergies in about 10% of patients and to also trigger FDEIA (54). According to data from European anaphylaxis registries, alcohol was reported as a cofactor of anaphylaxis even in up to 15.2% of patients (Table 1).

Like ASA, alcohol relaxes tight junctions in gut epithelium suggesting consecutive increase in intestinal protein absorption (25). Especially for small proteins, an alcohol-dependent increase in the intestinal absorption seems to be an underlying mechanism for anaphylaxis (55, 56); however, experimental evidence is still sparse.

Consequences for the clinician

To make the diagnosis of alcohol-triggered anaphylaxis, it is obligatory to perform oral provocation tests. Often, including additional cofactors such as ASA or exercise helps to confirm the diagnosis of alcohol-induced anaphylaxis.

Infections as cofactors of anaphylaxis

Clinical experience shows that especially early phases of infectious diseases and also clinically mild infections can effectively augment anaphylaxis. Next to case reports (10, 57), the best evidence showing a role of infectious diseases acting as cofactors of anaphylaxis comes from anaphylaxis registries, reporting a relevance of concomitant infection in 2.5–3% of anaphylactic reactions in children and in 1.3–11% in adults (Table 1). Most often, clinicians observed an association of infections and anaphylactic reactions following specific immunotherapy (SIT) with pollen or hymenoptera venoms. Although usually well tolerated, episodes of anaphylaxis occur after SIT in combination with infection and consequently patients undergoing SIT must not suffer from infections (5, 11, 57). In addition, the role of infections as cofactors of anaphylaxis is well documented in clinical trials with patients suffering from food allergy. Staden et al. (58) reported that 12 of 25 type I allergic children developed cofactor-induced anaphylaxis during oral tolerance induction with milk or eggs with the most common augmentation factor being ‘infection’ next to exercise.

Consequently, current guidelines for specific immunotherapy and manufacturers’ recommendations advise doctors to discontinue SIT in case of infection (11). Underlying mechanisms of how ‘infections’ act as cofactors of anaphylaxis are still not understood. Obviously and in contrast to other cofactors, provocation tests with and without infection as cofactor cannot be performed in humans and animal models suitable to elucidate the underlying mechanism on the cellular and molecular level were not yet established. Components of the ‘adaptive immune system’ were mostly addressed as mediators of enhanced type I allergy. Structures of pathogens leading to sensitization and IgE production or cross-reactivity with existing IgE could serve as allergens themselves (59–61). IgE antibodies, but even more important IgM and IgG antibodies, also form soluble multimeric antibody–antigen immune complexes. Under physiological conditions, this is an important way of antigen elimination. However, if phagocytosis is insufficient, immune complexes can cause damage by initiating complement activation resulting in formation of ‘anaphylatoxins’ C3a and C5a and other proinflammatory and chemotactic components (62). Besides FcεRI, basophils and mast cells also express activating FcγR, which can promote degranulation (63). Indeed, FcγRI-dependent degranulation of human mast cells mediated by antibodies of the IgG1 subclass is very similar to FcεRI stimulation in regard to the amount and the pattern of released mediators (64, 65). Thus, IgG production induced by ‘infection’ could also determine the outcome of anaphylaxis.

Alternatively but not yet addressed in detail, components of the ‘innate immune system’ could take part in the elicitation of type I allergy. This concept is especially intriguing, because substances from bacteria, fungi, or viruses generally known as ‘pathogen-associated molecular patterns’ (PAMP)
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can directly bind to pathogen recognition receptors (PRR) leading to cell activation and immune modulation without the need of previous sensitization (66, 67). It is well established that mast cells and basophils express PRR and are activated by different PAMP (68, 69), which could alter their responsiveness (70). Indeed, it was shown that the PAMP peptidoglycan (PGN) can induce degranulation in human (71) and murine mast cells (72). In addition, some PAMP can also modulate and inhibit mast cell degranulation pointing toward a well-balanced system of innate mast cell stimulation (73). Pathogens also lead to activation of the complement system resulting in generation of the so-called anaphylatoxins C3a and C5a. Several studies could demonstrate that both C3a and C5a trigger histamine release from mast cells, C5a being much more potent than C3a (74). However, the activating role of anaphylatoxins is restricted to certain subpopulations of mast cells because mucosal mast cells fail to express anaphylatoxin receptors (75) and their contribution to anaphylaxis is still unclear (76). In summary, bacterial or viral products can be sensed by receptors on mast cells and basophils and—under certain conditions—trigger or enhance mast cell degranulation. A model allowing to investigate how microbial factors act as cofactors of anaphylaxis is still lacking and research in this respect is ongoing.

Consequences for the clinician

Knowing about the role of infections as cofactors of anaphylaxis is most important in the context of SIT. SIT must be paused or continued with a reduced dose in case of infection. In case of infection-triggered anaphylaxis in a patient’s history, other cofactors like ASA or exercise should be used as surrogates in the test protocol.

Conclusion

The elicitation and severity of anaphylaxis depends on a variety of factors including the character of the allergen itself, the allergen dose, the sensitization status of the patient, and the affinity of the patient’s IgE for the respective allergens. In addition, it was increasingly recognized that co- or augmentation factors of anaphylaxis potentiate modulate the clinical response. Such cofactors of anaphylaxis are physical exercise, alcohol, NSAID, and infectious diseases (5). Strikingly, up to 39% of severe anaphylactic reactions are triggered by cofactors according to epidemiological studies with ‘physical exercise’ being the most frequent, followed by ‘alcohol’, ‘NSAID and other drugs’, and ‘infectious diseases’ (Table 1). Given the importance of cofactors for elicitation and severity of anaphylaxis, these cofactors need to be included into diagnostic measures and patients’ management. Provocation tests with or without cofactors are the gold standard for individual risk assessment. By definition, cofactors make patients become susceptible to lower allergen doses triggering anaphylaxis and increased allergen bioavailability or susceptibility to activation are believed to be the underlying causes (Table 2). Although we begin to understand the mechanisms underlying the most important cofactors of anaphylaxis, for a more complete understanding further research is urgently needed. Only the understanding of the processes leading to cofactor-triggered anaphylaxis will allow us to develop new and better treatments for those patients at risk and to give better advice to our patients.

Conflict of interest

There are no conflicts of interest according to the ICMJE Form for Disclosure of Potential Conflicts of Interest. The above-mentioned form was submitted for each author.

Author contributions

The article was written by Florian Wölling and Tilo Biedermann. Jörg Fischer, Martin Köberle and Susanne Kaesler each substantially contributed to the conception of the article as well as to acquisition, analysis, and interpretation of the data, critically revised the drafts of the article, and approved the final version for publication.

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