The role of IgG subclasses and platelets in experimental anaphylaxis

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In 1902, Charles Richet coined the term "anaphylaxis" to describe a "state of heightened sensitivity of a subject to a substance induced by a first injection, that instead of protecting the organism, renders it more fragile and more susceptible." Since this first description, experimental work led to identification of antibodies, receptors, cells, and mediators in this severe allergic reaction, leading to the paradigm that anaphylaxis is an IgE-dependent affliction that is triggered when allergens aggregate cognate IgE antibodies bound to the high-affinity IgE receptor (FcεRI) on the surface of mast cells and basophils. Their activation leads to the release of diverse bioactive mediators, including histamine, which are responsible for the associated clinical signs.[1] Seminal works from the Galli, Kinet, and Finkelman labs revealed, however, that anaphylaxis can also occur in mice deficient for IgE, FcεRI, or mast cells, and suggested that it could be driven by IgG antibodies engaging FcRγ-chain–containing receptors (reviewed in Reber et al[1]). Nowadays, the international consensus on anaphylaxis defines anaphylaxis as "a serious, generalized or systemic, allergic or hypersensitivity reaction that can be life-threatening or fatal." This deliberately generic definition excludes any precision on the pathophysiological mechanism involved.

In this article, we will discuss recent findings on IgG-dependent anaphylaxis with a focus on the role of IgG subclasses and platelets in these reactions.

IgG-DEPENDENT ANAPHYLAXIS IN WILD-TYPE MICE

IgG-dependent passive systemic anaphylaxis (IgG-PSA) can be elicited in mice by the transfer of specific IgG antibodies (of either IgG_1, $IgG_{2a/c}$, or IgG_{2b} subclass, but not IgG_3) followed by injection of their cognate antigen, or by transfer of precomplexed IgG (ie, immune complexes [IgG-ICs] or heat-aggregated IgG). IgG-PSA depends on IgG receptor (FcγR)-transduced activation of myeloid cells, leading to mediator release, which notably include platelet-activating factor (PAF).[1,2] Mice express 3 activating FcγRs (FcγRI, FcγRIII, and FcγRIV) and 1 inhibitory FcγR (FcγRIIB), each having a specific expression profile and distinct affinities for the different IgG subclasses. IgG-PSA in mice is associated with vasodilation, augmented vascular permeability, and a reduction in core temperature, motility, and awareness (Fig 1). Animals usually return to normal activity and behavior within 1 to 2 hours, but in rare cases cardiopulmonary failure results in death. (IgG)-anaphylaxis can also be triggered by antigen exposure of previously immunized mice that lack key players of the IgE-dependent pathway. IgG-independent immune players may however contribute to the reaction, rendering the interpretation of results more complex. To efficiently engage activating IgG receptors, IgG generally need to be present as multivalent complexes. There is a large consensus that IgG-PSA relies mainly on the engagement of FcγRIII and, to a lesser extent, on FcγRIV and possibly on FcγRI.[2,3]

The relative importance of FcγR-bearing effector cells to IgG-PSA remains more debated and is likely to depend on the experimental conditions.[1-3] Indeed, all cells of hematopoietic origin express at least 1 activating FcγR with the exception of T cells, B cells, and platelets, and could hence contribute to the reaction. Their involvement in anaphylaxis is often assessed either using depleting antibodies, which is problematic in the context of an antibody-dependent reaction, or using inhibitors, which may not be specific. In a comparative study using mouse IgG_1, IgG_{2a}, and IgG_{2b} with the same specificity to induce IgG subclass–specific anaphylaxis, we found that IgG_1 and IgG_{2b}-PSA shared a common mechanism that involved all tested myeloid cells and in which histamine H1 receptor blockade showed a stronger beneficial effect on PSA-associated temperature drop than PAF receptor blockade.[2] IgG_1- and IgG_{2b}-PSA were regulated by the inhibitory IgG receptor FcγRIIB present on all myeloid cells and B cells. In contrast, in IgG_{2a}-PSA, FcγRIIB-driven inhibition was negligible. IgG_{2a}-PSA was significantly reduced through depletion of neutrophils or monocytes/macrophages and attentuated by both PAF-receptor and histamine H1 receptor antagonists.[2] This particularity of IgG_{2a}-PSA may be due to the overall higher affinity of IgG_{2a} to FcγRs,[2] which also may explain the relative resistance of IgG_{2a}-PSA to changes in IgG/FcγR affinity induced by modification of IgG-glycosylation (ie, terminal sialylation) compared with IgG_1/IgG_{2b}-PSA.[4]

IgG ANAPHYLAXIS IN FcγR-HUMANIZED MICE

To approach human pathophysiology, anaphylaxis has been studied in mice carrying human FcγRs, either as a single

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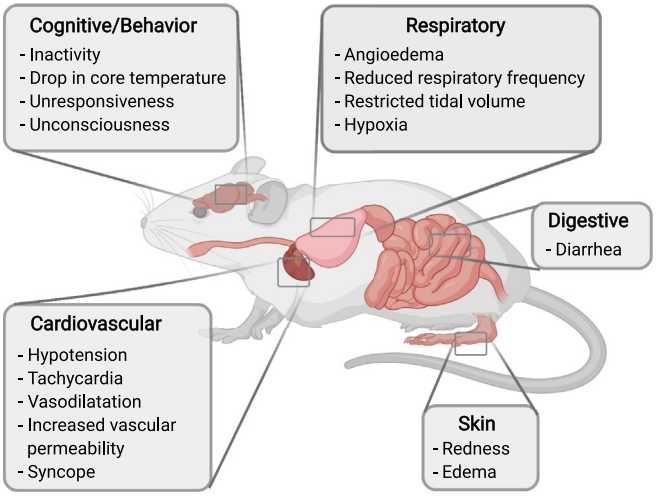

FIG 1. Pathophysiologic changes in experimental anaphylaxis in mice. Anaphylaxis is a systemic hypersensitivity reaction that affects multiple organs; the most common clinical signs of anaphylaxis in mice are indicated. Created with BioRender.com.

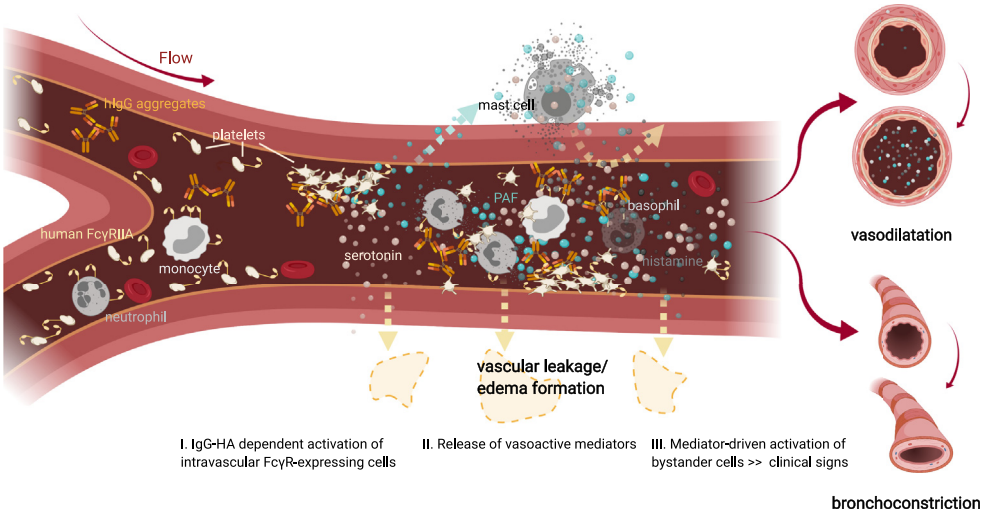

FIG 2. Model of IgG-dependent experimental anaphylaxis in hFcγRIIA-expressing mice in the absence of mouse endogenous FcγRs. Human FcγRIIA expression is conserved in hFcγRIIA-transgenic mice, including its expression on all myeloid cells and platelets. Injected heat-aggregated (HA)-IgG, mimicking IgG-ICs forming inside the circulation, can engage hFcγRIIA on any of these cells, but will have a stochastically higher likelihood to encounter platelets>neutrophils>monocytes>>basophils, leading to their activation. As a consequence, platelets will be activated, form aggregates, adhere to circulating leukocytes, and degranulate. Platelet-released serotonin can directly trigger anaphylaxis-associated vascular leakage, vasodilation, and bronchoconstriction. Platelet-released PAF, or PAF release by other IgG-IC–activated myeloid cells, can fuel the reaction through activation of perivascular mast cells, leading to histamine release. PAF and histamine may contribute to clinical signs of hFcγRIIA-PSA in mice. Created with BioRender.com.

transgene, as in the case of hFcγRIIA,[5-7] or in more complex models expressing several FcγRs.[6] Indeed, extrapolating results from IgG/FcγR-dependent reactions from mouse to human pathophysiology is challenging, because both species express very different sets of FcγRs (4 in mice and 6 in humans) that each shows distinct interaction profile with the different IgG subclasses (IgG_{1-4} in humans). Among human FcγRs, only FcγRIIB is inhibitory and expressed at much lower levels than in mice, suggesting that the regulation of IgG-driven reactions though coengagment of this inhibitory receptor remains limited in humans. Furthermore, as an example, whereas IgG_3 binds to all human FcγRs, its murine counterpart exclusively engages mouse FcγRI. These differences extend through all IgG subclasses, their affinities for FcγRs, and their capacity to trigger Fc-dependent effector functions. Studies in FcγR-humanized mice revealed that engagement of human FcγRs by IgG-ICs is sufficient to trigger anaphylaxis.[5] Among hFcγRs, hFcγRIIA appears to be the major contributor in IgG-PSA[6] and despite its expression on all myeloid cells, hFcγRIIA-expressing neutrophils and monocytes/macrophages, through their release of PAF, play a predominant role over mast cells, basophils, and eosinophils.[5] Unexpectedly, hFcγRIIA-transgenic mice also revealed the critical contribution of a blood

component that was until then overlooked in the context of anaphylaxis.

ROLE OF PLATELETS IN IgG ANAPHYLAXIS

Mouse platelets are devoid of any FcγR. Human platelets on the contrary express FcγRIIA/CD32A and incubation with IgG-ICs can induce their activation, aggregation, and release of granular content. Using hFcγRIIA-transgenic mice that confer IgG receptor expression to platelets, we and others demonstrated that IgG-induced platelet activation is critical for experimental anaphylaxis, and results in a rapid, severe, and prolonged (24-hour) thrombocytopenia.[6,7] Activated platelets released serotonin, which determined the severity of anaphylaxis.[6,7] Platelets also contributed to IgG-PSA in a more complex mouse model of cognate hFcγR expression.[6] Recently, platelet-released PAF was similarly proposed to trigger a transient disruption of endothelial integrity and mast cell activation resulting in shock.[8] Because of their abundance in blood, it is therefore conceivable that platelets are among the first players to become activated by circulating IgG-ICs, triggering a cascade of events that drives the activation and mediator release from various cell types contributing to anaphylaxis (Fig 2).

RELEVANCE FOR HUMAN ANAPHYLAXIS AND CONCLUSIONS

The fact that transgenic expression of a complete set of human FcγRs reproducing mostly the original expression profiles on all hematopoietic cells stimulated with human aggregated IgG is sufficient to induce anaphylaxis in mice[6] is a strong indicator for the relevance of IgG anaphylaxis in humans. Indeed, several lines of evidence support the existence of IgG/FcγR-, neutrophil-, and PAF-dependent human anaphylaxis. Cases of anaphylaxis were reported after administration of different therapeutic IgG antibodies,[1] and serum PAF concentrations correlate with anaphylaxis severity in humans.[9] In a clinical study of neuromuscular-blocking agent–induced anaphylaxis, concentrations of anti-drug IgG, markers of FcγR engagement and neutrophil activation (upregulation of CD11b and CD18, elevated levels of elastase and DNA-myeloperoxidase [MPO] complexes in the plasma), as well as reduced activity of PAF-acetyl hydrolase correlated with anaphylaxis severity.[10] Notably, neutrophil activation could be observed in patients lacking evidence of classical IgE-anaphylaxis.[10] Limited data from these patients further evidenced that platelet activation (upregulation of CD62P) was associated with anaphylaxis severity and that anaphylaxis occurrence was accompanied by a reduction in circulating platelet numbers.[6] These findings open new perspectives for the understanding and management of IgE-independent anaphylaxis in humans. In addition to certain drugs that can directly activate mast cells (notably through the recently described MRGPRX2), IgG-dependent reactions may account for or contribute to anaphylaxis, in particular when large amounts of IgG-ICs can form in the circulation. Further clinical studies will allow to determine whether it could be beneficial for patients at risk of developing IgG-driven anaphylaxis (ie, programmed intravenous administration of certain antibodies or drugs) to transiently receive treatments to block FcγRs (especially FcγRIIA) or limit the biological effects of serotonin and/or PAF.

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