Pathways of immediate hypothermia and leukocyte infiltration in an adjuvant-free mouse model of anaphylaxis

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Background: Conflicting results have been obtained regarding the roles of Fc receptors and effector cells in models of active systemic anaphylaxis (ASA). In part, this might reflect the choice of adjuvant used during sensitization because various adjuvants might differentially influence the production of particular antibody isotypes.

Objective: We developed an "adjuvant-free" mouse model of ASA and assessed the contributions of components of the "classical" and "alternative" pathways in this model.

Methods: Mice were sensitized intraperitoneally with ovalbumin at weekly intervals for 6 weeks and challenged intraperitoneally with ovalbumin 2 weeks later.

Results: Wild-type animals had immediate hypothermia and late-phase intraperitoneal inflammation in this model. These features were reduced in mice lacking the IgE receptor FcεRI, the IgG receptor FcγRIII or the common γ-chain FcRγ. FcγRIV blockade resulted in a partial reduction of inflammation without any effect on hypothermia. Depletion of monocytes/macrophages with clodronate liposomes significantly reduced the hypothermia response. By contrast, depletion of neutrophils or basophils had no significant effects in this ASA model. Both the hypothermia and inflammation were dependent on platelet-activating factor and histamine and were reduced in 2 types of mast cell (MC)–deficient mice. Finally, engraftment of MC-deficient mice with bone marrow–derived cultured MCs significantly exacerbated the hypothermia response and restored inflammation to levels similar to those observed in wild-type mice.

Conclusion: Components of the classical and alternative pathways contribute to anaphylaxis in this adjuvant-free model, with key roles for MCs and monocytes/macrophages. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Rodents, mouse model, mast cells/basophils, monocytes/macrophages, neutrophils, antibodies, Fc receptors, allergy, inflammation, anaphylaxis*

Anaphylaxis is an acute, life-threatening systemic allergic reaction with a lifetime prevalence of 0.05% to 2.0% in developed countries.[1-3] In human subjects it is largely accepted that anaphylaxis can be triggered by histamine and other mediators released in response to antigen cross-linking of IgE bound to its high-affinity receptor, FcεRI, on mast cells (MCs).[1-3] However, IgG might also contribute to anaphylaxis in some patients, especially those treated with infused drugs, such as therapeutic mAbs.[4,5]

Several mouse models of anaphylaxis have been developed and used to assess the contributions of IgE and IgG antibodies and the

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Abbreviations used
ASA: Active systemic anaphylaxis
BMCMC: Bone marrow–derived cultured mast cell
Cpa3: Carboxypeptidase A3
DT: Diphtheria toxin
DTR: Diphtheria toxin receptor
MC: Mast cell
Mcpt: Mast cell protease
OVA: Ovalbumin
PAF: Platelet-activating factor
PAF-AH: Platelet-activating factor acetylhydrolase
PCMC: Peritoneal cell–derived mast cell
PI: Propidium iodide
PSA: Passive systemic anaphylaxis
WT: Wild-type

roles of various effector cells and mediators. The analysis of passive local anaphylaxis or passive systemic anaphylaxis (PSA) models has allowed identification of 2 major pathways of anaphylaxis in mice: a "classical" pathway consisting of IgE, FcεRI, MCs, and histamine[6-8] and an "alternative" pathway consisting of IgG, FcγRIII and/or FcγRIV,[5,9-11] platelet-activating factor (PAF),[12,13] and, depending on the exact model used, macrophages,[5,14] basophils,[5,13] and/or neutrophils.[5,11,15]

Active systemic anaphylaxis (ASA) models, which are arguably more reflective of the clinical situation, have generated more conflicting results. Some ASA models critically depend on IgE, FcεRI, and MCs,[16-19] whereas some others can develop, at least with respect to the features analyzed, in $IgE^{-/-}$, $Fc\varepsilon RI^{-/-}$, and/or MC-deficient mice.[9,13,20] Depending on the mouse strain and ASA model used, basophils have been shown to either contribute to the systemic response[13,19] or play little to no significant role.[17,21] Similarly, depletion of monocytes/macrophages or antibody-mediated neutrophil depletion reduces anaphylaxis in some but not all ASA models.[11,17,19,21]

We hypothesize that such conflicting results in ASA models might reflect, at least in part, the choice of adjuvant used during sensitization because various adjuvants might differentially influence the production of individual antibody isotypes. Moreover, in human subjects with anaphylactic reactivity, sensitization to antigen generally occurs in the absence of an artificial adjuvant. Therefore we developed an "adjuvant-free" mouse model of ASA and assessed the contributions of components of the classical and alternative pathways of anaphylaxis in this model.

METHODS

Mice

C57BL/6J (wild-type [WT]) mice were purchased from Jackson Laboratories (Bar Harbor, Me) or Charles River (Lyon, France). $Fc\gamma RIII^{-/-}$ mice (B6.129P2-*Fcgr3*^{tm1Sjv}/J mice backcrossed to C57BL/6 for 12 generations) were purchased from Jackson Laboratories. $FcR\gamma^{-/-}$ mice (B6.129P2 *Fcer1g*^{tm1Rav} mice backcrossed to C57BL/6 for 12 generations) were from Taconic (New York, NY). C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice were originally provided by Peter Besmer (Memorial Sloan-Kettering Cancer Center, New York, NY); we then backcrossed these mice to C57BL/6J mice for more than 11 generations.[22] $Fc\varepsilon RI^{-/-}$ mice[7] (FcεRI α chain–deficient mice backcrossed to C57BL/6 for more than 8 generations and kindly provided by Jean-Pierre Kinet, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Mass), mast cell protease *(Mcpt8)*^{DTR} mice (backcrossed to C57BL/6 for at least 10 generations),[23] and carboxypeptidase A3 *(Cpa3)-Cre; Mcl-1*$^{fl/fl}$ mice (backcrossed to C57BL/6 for at least 9 generations)[24] were bred and maintained at the Stanford University Research Animal Facility. Age-matched male mice were used in all experiments. Except as otherwise noted, we used littermate controls in all experiments. All animal care and experimentation was conducted in compliance with the guidelines of the National Institutes of Health and specific approval of the Institutional Animal Care and Use Committee of Stanford University and the Animal Ethics Committee CETEA (Institut Pasteur, Paris, France) registered under #C2EA-89.

Ovalbumin-induced adjuvant-free model of active anaphylaxis

Six- to 8-week-old mice were sensitized intraperitoneally with 10 μg of endotoxin-free ovalbumin (OVA; Endograde OVA; BioVendor, Asheville, NC; <0.01 EU endotoxin per injection) in 100 μL of PBS once a week for 6 weeks. Two weeks after the last intraperitoneal sensitization, mice were challenged intraperitoneally with 500 μg of OVA. Rectal measurements of body temperature were performed immediately before (time 0) and at different time points for up to 2 hours after challenge. Mice were killed at various time points after challenge (as indicated) for assessment of inflammatory cell numbers in the peritoneal cavity and histology.

Other methods

See the Methods section in this article's Online Repository at www.jacionline.org for the methods for flow cytometry; peritoneal lavage; differential cell counts; depletion of basophils, monocytes/macrophages, and neutrophils; blockade of FcγRIV; histologic analysis; measurement of serum OVA-specific IgG_1 and IgG_{2c} antibodies; IgE-mediated PSA; ASA with adjuvant; treatment with an H_1-antihistamine or a PAF receptor antagonist; quantification of histamine and platelet-activating factor acetylhydrolase (PAF-AH); and generation and adoptive transfer of bone marrow–derived cultured mast cells (BMCMCs).

Statistical analyses

Results represent means ± SEMs or means + SEMs, with values for individual mice represented for quantifications of histamine, PAF-AH, and leukocytes. We used an unpaired Student *t* test (body temperature) or an unpaired Mann-Whitney *U* test (all other data) to assess the significance of differences between 2 sets of data. *P* values of less than .05 are considered statistically significant.

RESULTS

Development of an adjuvant-free model of ASA in C57BL/6 mice

We developed an adjuvant-free mouse model of ASA consisting of intraperitoneal sensitization with endotoxin-free OVA once a week for 6 weeks and intraperitoneal challenge with OVA 2 weeks after the last sensitization (Fig 1, *A*). OVA-sensitized C57BL/6 mice had hypothermia after OVA challenge that was maximal at 30 minutes and decreased thereafter (Fig 1, *B*). Sensitized and challenged mice also had a late-phase inflammatory response with increased numbers of total cells, eosinophils, macrophages, and lymphocytes in the peritoneal cavity, with the highest numbers of leukocytes on day 3 after challenge (Fig 1, *C-H*). Consistent with the increase in eosinophil numbers, we detected significant amounts of IL-5 in the plasma of 3 of 5 OVA-sensitized mice 18 (but not 72) hours after challenge, whereas levels of IL-4, IL-6, IL-10, TNF-α, and IFN-γ were all less than the detection limit of standard ELISAs at both time points (data not shown).