Plasma contact system activation drives anaphylaxis in severe mast cell–mediated allergic reactions

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Background: Anaphylaxis is an acute, potentially lethal, multisystem syndrome resulting from the sudden release of mast cell–derived mediators into the circulation.

Objectives and Methods: We report here that a plasma protease cascade, the factor XII–driven contact system, critically contributes to the pathogenesis of anaphylaxis in both murine models and human subjects.

Results: Deficiency in or pharmacologic inhibition of factor XII, plasma kallikrein, high-molecular-weight kininogen, or the bradykinin B2 receptor, but not the B1 receptor, largely attenuated allergen/IgE-mediated mast cell hyperresponsiveness in mice. Reconstitutions of factor XII null mice with human factor XII restored susceptibility for allergen/IgE-mediated hypotension. Activated mast cells systemically released heparin, which provided a negatively charged surface for factor XII autoactivation. Activated factor XII generates plasma kallikrein, which proteolyzes kininogen, leading to the liberation of bradykinin. We evaluated the contact system in patients with anaphylaxis. In all 10 plasma samples immunoblotting revealed activation of factor XII, plasma kallikrein, and kininogen during the acute phase of anaphylaxis but not at basal conditions or in healthy control subjects. The severity of anaphylaxis was associated with mast cell degranulation, increased plasma heparin levels, the intensity of contact system activation, and bradykinin formation.

Conclusions: In summary, the data collectively show a role of the contact system in patients with anaphylaxis and support the hypothesis that targeting bradykinin generation and signaling provides a novel and alternative treatment strategy for anaphylactic attacks. (J Allergy Clin Immunol 2015;135:1031-43.)

Key words: *Anaphylaxis, mast cell, bradykinin, mouse models, tryptase, contact system*

Anaphylaxis is a severe and often unanticipated multisystem syndrome of rapid onset and potentially fatal outcome. It most often represents an immunologic response to certain allergens, resulting in sudden systemic degranulation of mast cells and basophils.[1-3] Common triggers of anaphylaxis include food, medications, insect venoms, and other allergens cumulatively affecting as much as 1% to 15% of the population, with an increasing prevalence.[4] Anaphylaxis typically manifests with a broad range of symptoms, such as hypotension, vascular leakage, cardiac arrhythmias, bronchial constriction, and gastrointestinal and skin manifestations. Anaphylactic shock represents the most dramatic and potentially catastrophic manifestation of immediate hypersensitivity[5] and might account for more than 500 deaths annually in the United States.[6] Although the mechanisms have not been completely elucidated, systemic hypotension and circulatory shock are believed to result from peripheral vasodilatation, enhanced vascular permeability, plasma leakage, and intravascular volume depletion rather than a direct effect on the myocardium.[7] Despite recent advances in our understanding of the mechanisms and mediators involved in anaphylaxis, its typically acute and unforeseen presentation often hampers treatment.

In most cases anaphylaxis is initiated by an antigen (allergen) interacting with allergen-specific IgE bound to FcεRI on mast cells, basophils, or both; however, other immunologic and nonimmunologic mechanisms exist.[5] The allergen/IgE complex

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Abbreviations used
ACE: Angiotensin-converting enzyme
Anti-Xa: Anti–factor Xa activity
aPTT: Activated partial thromboplastin time
Bdkrb1: B1R coding gene
Bdkrb2: B2R coding gene
B1R: Bradykinin B1 receptor
B2R: Bradykinin B2 receptor
C1INH: C1 esterase inhibitor
DNP-HSA: Dinitrophenyl–human serum albumin
FXI: Factor XI
FXII: Factor XII
FXIIa: Activated factor XII
HAE: Hereditary angioedema
HK: High-molecular-weight kininogen
IQR: Interquartile range
Klkb1: Plasma kallikrein coding gene
Kng1: High-molecular-weight kininogen coding murine gene
MABP: Mean arterial blood pressure
NO: Nitric oxide
PCK: H-D-Pro-Phe-Arg-chloromethyl ketone
PK: Plasma kallikrein
PRCP: Prolylcarboxypeptidase
WT: Wild-type

initiates intracellular signaling that results both in release of preformed but also *de novo* synthesis of mediators, enzymes, and cytokines, including leukotrienes, histamine, the proteases tryptase and chymase, carboxypeptidase A, and proteoglycans. These highly sulfated polysaccharides, with heparin as the major component on a weight basis, are abundant in mast cell secretory granules and released on degranulation. *In vivo* heparin is exclusively synthesized in mast cells and contributes to the morphology and storage capacity of their secretory granules.[2] Purified and activated mast cell–released heparin provides the negatively charged surface for binding of the plasma protease factor XII (FXII; Hageman factor). Binding to a surface induces a conformational change in zymogen FXII, a process known as autoactivation (contact activation). Activated FXII (FXIIa) activates plasma kallikrein (PK) zymogen to the active protease, which in turn proteolytically generates the peptide hormone bradykinin from its precursor, high-molecular-weight kininogen (HK).[8] Bradykinin acts on G protein–coupled bradykinin B2 receptors (B2Rs) to increase vascular permeability.[9] Fig 6 shows a schematic overview of the FXIIa-driven contact system reaction cascade. Mast cell heparin triggers FXIIa-mediated bradykinin formation in human plasma *in vitro*[10,11] and increases vascular permeability in genetic mouse models of anaphylaxis.[12] Experimentally induced reactive nasal airway allergy locally increases bradykinin levels,[13-15] and bradykinin levels are increased in patients with allergic rhinitis.[16] Together the data suggest a role for bradykinin in local allergic reactions; however, clinical evidence for systemic activation of the contact system and ways to incorporate targeting bradykinin in the therapeutic management of anaphylaxis have remained underdeveloped.[17]

Here we analyze a potential role of the plasma contact system for anaphylaxis *in vivo* using a combination of contact system protein–deficient and humanized animal models and diseased patient plasma. The study shows a role of contact system–produced bradykinin in severe hypersensitivity reactions and suggests targeting bradykinin generation and its downstream signaling as a promising strategy for interference with anaphylaxis and possibly other immunologic disorders.

METHODS

Patients

We included all adult (≥18 years old) patients with anaphylaxis treated at the Department of Internal Medicine, Allergy Section of the University Hospital Vall d'Hebron, Barcelona, Spain, between June and August 2011. Only patients who fulfilled the definition of anaphylaxis with an allergy work-up that confirmed the diagnosis and ruled out other disease, had at least a serum and plasma sample taken during the episode and at baseline, and signed an informed consent form were included. We defined anaphylaxis according to the 2006 National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network criteria.[18] Anaphylaxis severity was classified according to the grading system based on clinical symptoms, as previously described,[19] according to the Brown classification (Table I).[20] The moderate group was subdivided into moderate A (grade 1, with gastrointestinal symptoms) and moderate B (grade 2, with respiratory symptoms). Grade 3 classifies severe anaphylaxis with hypotension.

Patients were followed up at the outpatient clinic of Vall d'Hebron University Hospital Allergy Section, where an allergy work-up was performed as needed (skin prick tests, specific IgE measurements, and/or challenge tests) and the diagnosis of anaphylaxis was confirmed by an allergist. Conditions that mimic anaphylaxis (eg, anxiety disorders, vocal cord dysfunction, or mastocytosis) were ruled out in all patients. We also obtained control plasma and serum samples from 10 age- and sex-matched patients, 4 atopic (3 with rhinitis and asthma caused by house dust mites and 1 with a history of urticaria caused by food allergy to peach) and 6 nonatopic patients, seen at the outpatient clinic of the same hospital between June and August 2011 for follow-up and treatment. All control subjects were asymptomatic, with no reported signs of allergy at the time of sampling. The ethics committee of Vall d'Hebron University Hospital approved the study (PR 53/2009), and all samples were collected with signed informed consent of the participants.

Animals

All animal care and experimental procedures complied with the Principles of Laboratory and Animal Care established by the National Society for Medical Research and were approved by the Bezirksregierung of Unterfranken or Stockholm's Norra Djurförsöksetiska Nämnd. *F12*$^{-/-}$, *FXI*$^{-/-}$, *Bdkrb1*$^{-/-}$, *Kng1*$^{-/-}$, and *Bdkrb2*$^{-/-}$ mice were backcrossed for more than 10 generations to the C57Bl/6 background, as previously described.[21,22] All progeny were genotyped by using PCR. All studies were performed on male mice 6 to 8 weeks of age. Age- and sex-matched wild-type (WT) control mice were purchased from Charles River (Wiga, Sulzfeld, Germany).

Generation of *Klkb1*$^{-/-}$ mice, genotyping, and expression analysis

The *Klkb1*$^{-/-}$ mice were generated by using a homologous recombination-based targeting strategy that replaces exon 1 (bp 1549 to 1567 in the murine PK gene of 2573 bp) with a Neo cassette. Successfully targeted C57Bl/6 embryonic stem cells were identified by means of Southern blotting of *Eco*RV/*Spe*I-digested isolated DNA from embryonic stem cells by using a probe with labeled DNA directed just outside the construct arm. We used genomic DNA from tail samples for PCR genotyping under the following conditions: denaturation at 94°C for 15 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 40 seconds. The PCR was run in 30 cycles with the following 3 primers for genotyping of WT-specific product: 5′-CCAATGTGACTCGTTTCCTGACTTG-3′, 5′-GATCCTAGTTGGGGAGCCATCTGTG-3′, and 5′-GGGTGGGATTAG ATAAATGCCTGCTCT-3′, which amplify fragments of 567 and 365 bp