The vascular endothelial specific IL-4 receptor alpha–ABL1 kinase signaling axis regulates the severity of IgE-mediated anaphylactic reactions

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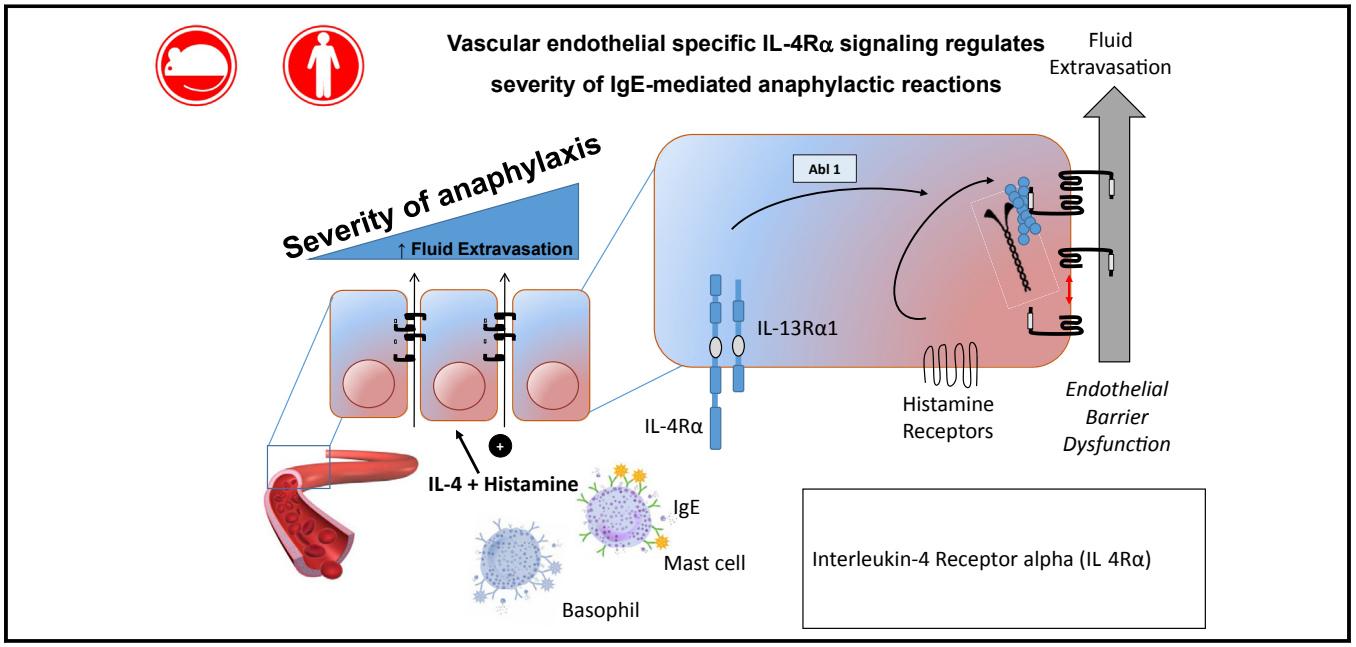
GRAPHICAL ABSTRACT



Background: Severe IgE-mediated, food-induced anaphylactic reactions are characterized by pulmonary venous vasodilatation and fluid extravasation, which are thought to lead to the life-threatening anaphylactic phenotype. The underlying immunologic and cellular processes involved in driving fluid extravasation and the severe anaphylactic phenotype are not fully elucidated.

Objective: We sought to define the interaction and requirement of IL-4 and vascular endothelial (VE) IL-4 receptor α chain (IL-4Rα) signaling in histamine-abelson murine leukemia viral oncogene homology 1 (ABL1)–mediated VE dysfunction and fluid extravasation in the severity of IgE-mediated anaphylactic reactions in mice.

Methods: Mice deficient in VE IL-4Rα and models of passive and active oral antigen– and IgE-induced anaphylaxis were used to define the requirements of the VE IL-4Rα and ABL1 pathway in severe anaphylactic reactions. The human VE cell line (EA.hy926 cells) and pharmacologic (imatinib) and genetic (short hairpin RNA knockdown of *IL4RA* and *ABL1*) approaches were used to define the requirement of this pathway in VE barrier dysfunction.

Results: IL-4 exacerbation of histamine-induced hypovolemic shock in mice was dependent on VE expression of IL-4Rα. IL-4– and histamine-induced ABL1 activation in human VE cells and VE barrier dysfunction was ABL1-dependent. Development of severe IgE-mediated hypovolemia and shock required VE-restricted ABL1 expression. Treatment of mice with a history of food-induced anaphylaxis with the ABL kinase inhibitor imatinib protected the mice from severe IgE-mediated anaphylaxis.

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Supported by National Institutes of Health grants R01 AI073553, R01 AI 112626, R01 DK090119 (to S.P.H.), P30DK078392, and U19A1070235 and a Food Allergy Research Education Award (to S.P.H.).

Disclosure of potential conflict of interest: A. Yamani receives funding from grants from the National Institutes of Health (NIH) and Food Allergy Research & Education (FARE). D. Wu receives funding from grants from the NIH and FARE. L. Waggoner receives funding from grants from the NIH and FARE. T. Noah receives funding from rants from the NIH and FARE. A. J. Koleske receives funding from grants from the NIH and FARE. F. Finkelman receives fees or funding for consultancy from Vedanta Bioscience, is employed by the University of Cincinnati and Fress Therapeutics, and receives grants from NIH, FARE, the University of Cincinnati, Cincinnati Children's Hospital. S. P. Hogan receives funding from grants from the NIH and FARE.

Received for publication May 19, 2017; revised August 4, 2017; accepted for publication August 31, 2017.

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0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2017.08.046

Conclusion: IL-4 amplifies IgE- and histamine-induced VE dysfunction, fluid extravasation, and the severity of anaphylaxis through a VE IL-4Rα/ABL1–dependent mechanism. These studies implicate an important contribution by the VE compartment in the severity of anaphylaxis and identify a new pathway for therapeutic intervention of IgE-mediated reactions. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *IL-4 receptor α chain, hypovolemic shock, IgE and mast cells, food-induced anaphylaxis, histamine, vascular endothelial barrier dysfunction, ABL1 kinase*

Anaphylaxis is a severe, life-threatening allergic reaction that affects both children and adults and male and female subjects in the United States.[1] The most common inciting agents (33.2% of reactions) are foods, particularly peanuts and tree nuts, and food-induced anaphylaxis hospitalization rates for children in the United States have more than doubled from 2000 to 2009.[2]

A food-induced anaphylactic reaction encompasses a variety of symptoms that can affect 1 or more target organs, including those of the gastrointestinal, cutaneous, respiratory, and cardiovascular systems.[3-5] In human subjects compromise of either the cardiovascular or respiratory system defines a severe reaction,[3,4] and it is postulated that basophil- and mast cell (MC)–derived mediators, through inducing pulmonary venous vasodilatation and fluid extravasation, cause the respiratory and cardiovascular collapse that leads to the severe, life-threatening anaphylactic phenotype.[6] Fluid extravasation in patients with anaphylaxis is thought to be a consequence of capillary fluid leak caused by loss of vascular endothelial (VE) barrier integrity, leading to the movement of fluids, electrolytes, and proteins from the vascular compartment into the interstitial spaces.[7-9]

The VE barrier is maintained by adherens junction (AJ) and tight junction (TJ) proteins.[10] The AJ proteins are the most ubiquitously expressed endothelial cell-cell junctional proteins and act as mechanical anchoring points that permits endothelial TJ protein-protein interactions and interjunctional integrity.[11] The TJ proteins are tethered to the actin cytoskeleton and seal the intercellular space, establishing the dense "fence" barrier preventing the bilateral apical-basolateral passage of ions, proteins, and lipids.[11] VE-cadherin is one of the first endothelial cell–specific molecules expressed and is required for endothelial survival, blood vessel assembly, and stabilization.[12,13] VE-cadherin forms Ca^{2+}-dependent homophilic interactions with adjacent endothelial cells through actin-linking catenin family proteins and the actin cytoskeleton, establishing the vascular barrier integrity.[11] The stability of the VE-cadherin–catenin–cytoskeleton complex is essential to maintaining endothelial barrier function.[14] Disruption of these processes through receptor-signaling pathways, including nonreceptor kinases, such as Src abelson murine leukemia viral oncogene homology 1 (ABL1), and Arg and myosin light chain kinase, leads to VE-cadherin–mediated AJ disorganization or VE-cadherin internalization and loss of endothelial barrier integrity.[14-20]

The cellular and molecular pathways that directly contribute to the severe anaphylaxis phenotype are unclear. Clinical studies have reported increased levels of IL-4 and histamine in the sera of human patients with severe anaphylaxis.[21] While histamine, but not IL-4 levels were associated with severe disease,[22] there is likely involvement of both of these molecules in expression of the severe disease phenotype. We and others have demonstrated that symptoms of food-induced anaphylaxis in mice are dependent on IgE/MC and histamine type I receptor signaling and that the severity of the reaction positively correlates with an increase in hemoconcentration (an indication for fluid extravasation and hypovolemic shock).[23-26] *In vitro* experimental evidence suggests that IL-4 modulates VE barrier properties,[27-29] and we have demonstrated that IL-4 can interact with vasoactive mediators to increase hemoconcentration and the severity of anaphylaxis.[30,31] However, the cellular target of these IL-4–mediated effects and the underlying IL-4 receptor α chain (IL-4Rα)–dependent signaling processes involved in the amplification of histamine-induced VE barrier dysfunction and fluid extravasation in IgE-mediated reactions are not yet fully understood.

Here we examine the relationship between IL-4 and histamine in IgE-mediated VE leak and hypovolemic shock. We show that IL-4 amplifies histamine-induced hypovolemic shock through VE IL-4Rα chain–dependent process. Furthermore, we show that IL-4 and histamine stimulate ABL1 kinase activity in VE cells and VE barrier dysfunction was inhibited by pharmacologic and genetic ablation of ABL1 activity. Importantly, by using both passive and active models of food-induced anaphylaxis, treating mice with the ABL kinase inhibitor imatinib protected the mice from the severe IgE-mediated anaphylactic phenotype after allergen exposure. These studies implicate an important contribution by the IL-4Rα/ABL1 signaling pathway in the VE compartment in the severity of IgE- and histamine-induced anaphylaxis.

Abbreviations used

ABL1:	Abelson murine leukemia viral oncogene homology 1
AJ:	Adherens junction
CCHMC:	Cincinnati Children's Hospital Medical Center
HRP:	Horseradish peroxidase
iIL-9Tg:	Intestinal IL-9 transgenic mice
IL-4C:	IL-4 complex
IL-4Rα:	IL-4 receptor α chain
LB:	Luria broth
MC:	Mast cell
mMCPT-1:	Mouse mast cell protease 1
OVA:	Ovalbumin
shRNA:	Short hairpin RNA
TER:	Transendothelial resistance
TJ:	Tight junction
TKI:	Tyrosine kinase inhibitor
TNP:	2,4,6-Rrinitrophenol
VE:	Vascular endothelial
WT:	Wild-type

METHODS

Animals

Intestinal IL-9 transgenic (iIL9Tg) mice were generated, as previously described.[32] Wild-type (WT) BALB/c mice were originally provided by Charles River laboratories (Wilmington, Mass) and bred in-house at Cincinnati Children's Hospital Medical Center (CCHMC; Cincinnati, Ohio). IL-4Rα^{Y709F} mice were obtained from Fred Finkelman (CCHMC).[33] Cadherin-5^{Cre} mice (purchased from Jackson Laboratory, Bar Harbor, Me), IL-4Rα$^{fl/fl}$ mice (generously provided by Frank Brombacher, University of Cape Town, South Africa), and iIL9Tg mice were used to generate mice lacking IL-4Rα in VE cells.[34] Tie2^{Cre} mice (generously provided by Joseph E. Qualls, CCHMC), Abl1$^{fl/fl}$ mice,[35] and iIL-9Tg mice were used to generate mice lacking ABL1 in VE cells.[36] Age-, sex-, and weight-matched littermates