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Invited review article

Mast cells and IgE in defense against venoms: Possible “good side” of allergy?*

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Abbreviations:

ABS, rabbit anti-basophil serum; AES, rabbit

anti-eosinophil serum; BMCs, bone

marrow-derived cultured mast cells;

BV, honeybee venom; bvPLA₂, honeybeevenom phospholipase A₂;

CPA3, carboxypeptidase A3; CBH, cutaneous

basophil hypersensitivity;

DNP, dinitrophenol; DNP-

HSA, dinitrophenol-conjugated human

serum albumin; ESCMCs, embryonic stem

cell-derived cultured mast cells; ET-

1, endothelin-1; F(ab), antigen-binding

fragment of an immunoglobulin molecule;

FcεRI, the high affinity receptor for IgE;

IgE, immunoglobulin E (antibody);

IgG, immunoglobulin G (antibody);

IL, interleukin; ILC2, innate lymphoid cells,

type 2; i.d., intradermal;

i.p., intraperitoneal; i.v., intravenous;

LPS, lipopolysaccharide; MC(s), mast cell(s);

Mcl-1, myeloid cell leukemia 1; MCP4, mast

cell protease 4; NRS, normal rabbit serum;

PAMPs, pathogen-associated molecular

patterns; RVV, Russell's viper venom;

ABSTRACT

Physicians think of mast cells and IgE primarily in the context of allergic disorders, including fatal anaphylaxis. This ‘bad side’ of mast cells and IgE is so well accepted that it can be difficult to think of them in other contexts, particularly those in which they may have beneficial functions. However, there is evidence that mast cells and IgE, as well as basophils (circulating granulocytes whose functions partially overlap with those of mast cells), can contribute to host defense as components of adaptive type 2 immune responses to helminths, ticks and certain other parasites. Accordingly, allergies often are conceptualized as “misdirected” type 2 immune responses, in which IgE antibodies are produced against any of a diverse group of apparently harmless antigens, as well as against components of animal venoms. Indeed, certain unfortunate patients who have become sensitized to venoms develop severe IgE-associated allergic reactions, including fatal anaphylaxis, upon subsequent venom exposure. In this review, we will describe evidence that mast cells can enhance innate resistance to reptile or arthropod venoms during a first exposure to such venoms. We also will discuss findings indicating that, in mice which survive an initial encounter with venom, acquired type 2 immune responses, IgE antibodies, the high affinity IgE receptor (FcεRI), and mast cells can contribute to acquired resistance to the lethal effects of both honeybee venom and Russell's viper venom. These findings support the hypothesis that mast cells and IgE can help protect the host against venoms and perhaps other noxious substances.

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s.c., subcutaneous; shRNA, small hairpin RNA; Th2, T helper cell type 2; VIP, vasoactive intestinal polypeptide; WT, wild type

Mast cells, basophils and IgE in the pathology of allergic disorders

Allergies, that currently afflict 20–30% of people worldwide, are detrimental immune responses against any of a large variety of environmental antigens.¹ Such antigens (called allergens) share the ability to elicit acquired type 2 immune responses which are orchestrated by CD4⁺ T helper type (Th)2 cells and include the production of allergen-specific IgE antibodies.^{2–4} In such Th2 cell-associated “type 2” immune responses, IgE enables antigen-specific function of effector cells by binding to high affinity receptors for IgE (FcεRI) on the cells’ surface.^{5,6} FcεRI are expressed on mast cells, that reside in most vascularized tissues in mammals and other vertebrates, and on basophilic granulocytes (“basophils”), that ordinarily circulate in very low numbers in the blood but which can be recruited to sites of inflammation.^{3,5–10}

When mast cell- or basophil-bound IgE antibodies recognize antigens that are at least bivalent, rapid aggregation of the FcεRI initiates complex intra-cellular signaling pathways. This ultimately results in the release, by such activated effector cells, of a wide variety of mediators with diverse biological effects.^{5,6,8–11} Some of these mediators are stored in the cells’ cytoplasmic granules, ready for immediate release, including, in mast cells, histamine, heparin and other proteoglycans, proteases such as carboxypeptidase A3 (CPA3), tryptases and chymases, and some cytokines; in addition, products of arachidonic acid metabolism (*via* the cyclo-oxidase or lipoxygenase pathways; e.g., prostaglandins and cysteinyl leukotrienes) and a diverse group of cytokines, chemokines and growth factors are secreted after upregulation of their transcription as a result of FcεRI-dependent cell activation.^{3,5–7,12,13} Basophils activated *via* FcεRI aggregation can release a panel of mediators partially overlapping with those of mast cells, but, as compared to mast cells, they contain much lower amounts of proteases and appear to produce fewer cytokines and chemokines.^{8–10}

Innate activation of mast cells

In addition to IgE and specific antigen, many stimuli can activate at least some mast cell populations *via* innate mechanisms, including products of complement activation (e.g., C3a, C5a), products of pathogens (e.g., LPS and other pathogen-associated molecular patterns [PAMPs]), certain cytokines or growth factors (including IL-33 and the Kit ligand, stem cell factor), products of other hematopoietic cells, certain endogenous peptides (including endothelin-1 [ET-1] and vasoactive intestinal polypeptide [VIP]), and components of the venoms of many different vertebrates and invertebrates.^{10,14–18} Within or among different mammalian species, individual mast cell subpopulations can vary in their susceptibility to activation *via* these innate mechanisms, likely reflecting such factors as microenvironmentally regulated differences in levels of expression of the cognate receptors.^{14,19} Also, various stimuli can differ in their ability to elicit the release of granule-stored, lipid, or cytokine mediators. For example, certain peptides such as substance P can activate some mast cell populations to robustly release the granule-stored mediators; however, compared to the same cells activated *via* the FcεRI, such stimuli may less potently elicit release of lipid mediators or cytokines.^{14,20,21} In contrast, for at least some mast cell populations, PAMPs are more

effective in eliciting release of cytokines and chemokines than granule-stored mediators.^{16,17} Because mast cells or basophils participating in innate or adaptive immune responses may encounter simultaneously or sequentially several different stimuli of activation, it may be difficult to predict which mast cell- or basophil-derived mediators will be released and in what amounts in these settings, and even more challenging to guess what the net effects of all such mediators might be during that particular biological response.

Possible beneficial functions of mast cells, basophils and IgE

It is now generally accepted that mast cells and basophils can contribute importantly to the pathology associated with allergic disorders, including potentially fatal anaphylaxis.^{3,22,23} Yet the evolutionary advantages which might be conferred by IgE, mast cells and basophils have been more difficult to define. A major hypothesis about the potential “beneficial functions” of such allergic effector mechanisms is that IgE-associated type 2 immune responses contribute to host defense against helminths and certain other parasites.^{4,24–26}

It should be noted, however, that it has been challenging to prove that IgE, mast cells or basophils dramatically influence the survival of parasite-infected animals. Abnormalities in host responses to certain parasites have been observed in mice that genetically lack IgE,^{27,28} mast cells,^{29–33} or basophils,^{28,33} but such studies generally have not included an analysis of the effects of those deficiencies on the overall survival or reproductive success of the infected hosts. And some findings even suggest that, in certain settings, IgE or mast cells may have effects during host responses to parasites (e.g., effects which directly or indirectly result in increased parasite egg production) that may favor the parasite rather than the host.^{34–36}

The complexity of the relationships between parasites and their hosts is not surprising, given that vertebrates have been co-evolving with such parasites for millions of years. It therefore also is not surprising that, depending on the parasites and the particular setting, immune effector mechanisms such as IgE, mast cells and basophils might be exploited by the parasites to their own advantage. For example, one can speculate that by eliciting a type 2 immune response that results in IgE-dependent mast cell activation and release of vasoactive mediators in response to parasite antigens at sites of parasite infection, the parasite could influence local blood flow and vascular permeability in ways that enhance the parasite’s nutrition.

In contrast to parasites, most allergens do not represent a direct threat to the non-sensitized host. This is why such type 2 immune responses are widely considered to be “misdirected” or “maladaptive” immune responses.^{37,38} However, Margie Profet proposed a radically different notion in 1991, based in part on the observation that the common feature of most allergens is their origin from sources such as seafood, nuts, or venoms which either might contain toxins (e.g., foods) or always do (e.g., venoms).³⁹ Profet proposed that acute allergic reactions, manifested as immediately occurring symptoms in response to allergen exposure, such as sneezing, coughing, vomiting and diarrhea, evolved as defense mechanisms allowing the sensitized host to respond immediately to, and to expel, neutralize and/or avoid, noxious substances which

might be indicative of potentially life-threatening situations.³⁹ Even before Profet's 1991 paper, James Stebbings, Jr. hypothesized that "a major function of the immediate hypersensitivity reactions has been the protection of terrestrial vertebrates from the bites of, or invasion by, arthropods".⁴⁰ However, until recently,⁴¹ Profet's "toxin hypothesis" was largely ignored by the scientific community; Stebbings' paper was even more neglected.⁴²

Evidence for a beneficial role of basophils in acquired immunity to the feeding of ixodid ticks

Galli et al. generated a rabbit anti-guinea pig basophil antiserum (ABS) and showed that intravenous (i.v.) administration of ABS markedly reduced numbers of blood basophils *in vivo*, without reducing numbers of blood eosinophils or the total white blood cell count, or changing numbers of intradermal mast cells.⁴³ ABS did cause a reduction in skin mast cell numbers if injected intradermally, raising the possibility that the antiserum targeted an antigen shared by basophils and mast cells. Administration of this ABS i.v. to guinea pigs sensitized to express "cutaneous basophil hypersensitivity" (CBH), an antigen-induced, delayed onset, erythematous skin reaction that contains large numbers of infiltrating basophils,⁴⁴ revealed that ABS could be used to reduce markedly the numbers of basophils infiltrating the skin at such sites.⁴³

Brown et al. then used this rabbit ABS, and a rabbit anti-eosinophil serum (AES), to investigate whether basophils or eosinophils contributed to adaptive immune response that diminish the feeding success of larval *Amblyomma americanum* ticks.⁴⁵ *A. americanum* (the Lone star tick) is an ixodid tick that is a vector for a variety of diseases including Rocky Mountain spotted fever (*Rickettsia rickettsia*), Q fever (*Coxiella burnetii*), tularemia (*Francisella tularensis*), granulocytic ehrlichiosis (*Ehrlichia ewingii*), monocytotropic ehrlichiosis (*Ehrlichia chaffeensis*), and others.⁴⁶ Moreover, Platts-Mills and colleagues recently demonstrated that the bites of this tick can induce the development of IgE antibodies to a tick carbohydrate, galactose- α -1,3-galactose, that is also present in non-primate meat and meat products, sensitizing such individual to develop a delayed form of urticaria or anaphylaxis after they consume such meats or meat products⁴⁷ or in response to treatment with a therapeutic antibody containing this carbohydrate.⁴⁸

It was known that when guinea pigs subjected to a single round of exposure to the feeding of larval *A. americanum* ticks are challenged with a second exposure to the larval ticks about a month later, tick feeding success is markedly diminished, and such acquired resistance can be transferred by administering serum from sensitized to naïve guinea pigs.⁴⁹ It also was known that tick feeding sites in sensitized guinea pigs contained large numbers of basophils and eosinophils.⁵⁰ Brown et al. found that treatment of tick-sensitized guinea pigs with rabbit ABS markedly diminished basophils in the bone marrow, blood, and skin at sites of tick feeding of the sensitized guinea pigs, and also resulted in diminished numbers of eosinophils at such feeding sites.⁴⁵ Notably, ABS treatment also essentially ablated the acquired resistance to tick feeding conferred by sensitization (Fig. 1)⁴⁵. Treatment with rabbit AES had a lesser, but still statistically significant, effect on tick feeding success, whereas normal rabbit serum was without effect (Fig. 1).⁴⁵

To our knowledge, this was the first *in vivo* experimental evidence that went beyond correlative and observational studies (i.e., demonstrating the presence of basophils at tick feeding sites) indicating that basophils might represent one important component of acquired, antibody-dependent resistance to the feeding of an arthropod. Years later, it was possible to pursue similar experiments in mice genetically deficient in either mast cells or basophils. In convincing studies, Hiroshi Matsuda and Yukihiko Kitamura and colleagues (using mast cell-engrafted genetically mast cell deficient (WB/Re-W/+ X C57BL/6-W^v/+)F₁-W/W^v mice),⁵¹ and Hajime Karasuyama and colleagues (using mice genetically deficient in basophils⁵²), provided evidence that both mast cells and basophils, as well as IgE, can contribute to acquired immunity to the feeding of *Haemaphysalis longicornis* ticks in mice. By contrast, it appears that basophils may have a more important role than mast cells in acquired resistance to the feeding of larval *Dermacentor variabilis* ticks in mice.⁵³

"Mast cell knock-in mice"

Yukihiko Kitamura and colleagues discovered that (WB/Re-W/+ X C57BL/6-W^v/+)F₁-W/W^v mice (now known as WBB6F₁-Kit^W/Kit^{W-v} mice, since "W" later was shown to encode c-kit^{54,55}) not only

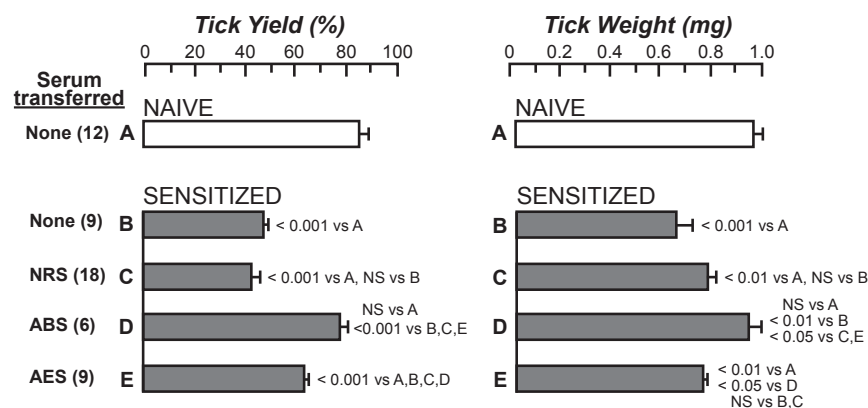


Fig. 1. Effects of treatment with anti-basophil serum (ABS), anti-eosinophil serum (AES) or normal rabbit serum (NRS) on feeding success of larval *Amblyomma americanum* ticks in a second infestation of guinea pigs. One hundred larval *Amblyomma americanum* ticks were placed on the flanks of "nonsensitized" (naïve) guinea pigs or guinea pigs which had been "sensitized" 26 days earlier by a primary infestation of *A. americanum*. The naïve guinea pigs (A) and one group of sensitized hosts (B) received no serum, other sensitized animals were treated with NRS (C), ABS (D), or AES (E) as described in⁴⁵. The number (Tick Yield, left) and weight (Tick Weight, right) of engorged ticks was determined at 90 h of infestation. ABS completely ablated immunity; AES partially impaired resistance; NRS had no effect. Data (mean $\pm$ SEM) were pooled from three separate experiments, with the total number of animals in each group shown in parentheses. NS = not significant ($P > 0.05$). Differences among the experimental groups were analyzed by the Newman-Keuls multiple sample comparison test. [This is a modified version of Fig. 2 in Brown SJ, Galli SJ, Gleich GJ, Askenase PW. Ablation of immunity to *Amblyomma americanum* by anti-basophil serum: cooperation between basophils and eosinophils in expression of immunity to ectoparasites (ticks) in guinea pigs. *J Immunol* 1982;129:790–6 (Ref. 45) reprinted with the permission of the publisher. Copyright 1982. The American Association of Immunologists, Inc.]

had a moderate macrocytic anemia, a phenotype which had been reported decades earlier, but were profoundly deficient in tissue mast cells.⁵⁶ They also showed that mast cells developed in WBB6F₁-W/W^v mice which had been engrafted with bone marrow cells from the wild type (WT) littermate WBB6F₁-+/+ mice.⁵⁶ However, the recipient W/W^v mice also were cured of their anemia,⁵⁶ as was initially shown by Elizabeth Russell.⁵⁷ Moreover, eventually, non-irradiated WBB6F₁-W/W^v mice engrafted with sufficient (e.g., 1×10^7) WBB6F₁-+/+ or other genetically-compatible WT whole bone marrow cells also undergo virtually complete replacement of multiple other hematopoietic lineages (including granulocytes and lymphocytes) with cells of donor origin.^{58–60}

Because transfer of WT bone marrow cells into W/W^v mice did not result in the selective engraftment of mast cells, due to the presence in bone marrow cells of hematopoietic stem and progenitor cell populations, an effort was undertaken to attempt to achieve a more selective “repair” of the mast cell deficiency of W/W^v mice by transferring *in vitro*-derived, “lineage-committed”, mast cells to the mice instead of whole bone marrow cells. This approach appeared plausible because it was clear that large populations of cells with features of “immature” mast cells could be generated *in vitro* from mouse hematopoietic progenitor cells⁶¹ and that such cells exhibited features of additional mast cell maturation when exposed to sodium butyrate *in vitro*.⁶² Nakano et al. showed that the transfer of WBB6F₁-+/+ mouse bone marrow-derived cultured mast cells (BMCMCs) i.v., i.p. or i.d. into WBB6F₁-W/W^v mice had no effect on the anemia of the recipient mice but resulted in the appearance of mast cells in their tissues, and that, over time, these mast cell populations came to exhibit certain phenotypic features similar to those present in the corresponding anatomical sites in WT mice.⁶³

Since that first study, many groups have used such mast cell-engrafted or, as we refer to them in the Galli lab, “mast cell knock-in mice” (Fig. 2), to analyze mast cell development, phenotype, heterogeneity and function *in vivo*.^{14,71,72} An attractive aspect of this approach is that one can transfer into different genetically mast cell-deficient recipients either WT mast cells or mast cells that have been genetically manipulated or that are derived from various mutant or transgenic mice, so that one can compare the function *in vivo* of mast cells that are normal or that lack (or have altered function of) various receptors, signaling molecules or mediators. Moreover, Tsai et al. showed that one also can generate such mast cells from embryonic stem cells, permitting the analysis *in vivo* of mast cells which lack products which, if absent in the germ line, would result in embryonic or perinatal lethality.⁶⁴

In addition to using the original mast cell-deficient WBB6F₁-Kit^W/Kit^{W-v} mice to prepare “mast cell knock-in mice”, this approach also can be employed using C57BL/6-Kit^{W-sh}/Kit^{W-sh} mice, which have the advantage of being inbred, fertile and not anemic.^{68,69,73} We recently showed that this approach also can be pursued using C57BL/6-Cpa3-Cre⁺-Mcl-1^{fl/fl} mice, which are mast cell-deficient (and also have substantially diminished numbers and function of basophils) due to the lineage-restricted ablation of the anti-apoptotic factor, myeloid cell leukemia 1 (Mcl-1), in lineages with sufficiently high expression of the Cpa3 gene.⁷⁰ Because the latter mice are mast cell deficient but, unlike WBB6F₁-Kit^W/Kit^{W-v} or C57BL/6-Kit^{W-sh}/Kit^{W-sh} mice, have normal c-kit, we informally call them “Hello Kitty” mice⁷⁰ (Fig. 2).

New models for analyzing the functions of basophils and mast cells

In addition to the models described above, those interested in the biology of mast cells, basophils or their mediators are now

fortunate to have a large number of additional models to choose from, including other lines of mice that exhibit constitutive or inducible deficiencies in populations of mast cells or in basophils, or which constitutively or inducibly lack various mast cell mediators or other molecules.⁷² As reviewed in detail elsewhere,^{72,74,75} each of the various models currently available has features that must be kept in mind when interpreting the results of studies using such mice, and the importance of particular mast cell (or basophil) roles in individual biological responses may vary both according to the details of the model used to study that biological response (e.g., whether one is studying a “weak” or “strong” model of that response) and based on the strain background of the mice. Accordingly, we have recommended that investigators consider using more than one type of genetic model to investigate the functions and importance of mast cells (or basophils) and/or their individual products in biological responses *in vivo*.⁷²

Identifying a beneficial role for mast cells in enhancing innate resistance to venoms

Early work by Higginbotham and colleagues suggested that mast cells might be able to diminish the toxicity of certain venoms by degranulating and releasing heparin in response to venom exposure.^{76,77} However, this work was conducted before the description of mice deficient in mast cells or their individual mediators, so the importance of the roles of mast cell and their products in innate resistance to venoms could not be investigated more definitively *in vivo*. Even after Kitamura's description of genetically mast cell-deficient mice, some time elapsed before any attention was paid to this question. One step in that direction was the finding that ET-1 can initiate a homeostatic mechanism whereby proteases released by mast cells activated by ET-1 can degrade that vasoactive peptide and thereby diminish its potential toxicity *in vivo*.⁶⁵ Using mast cell knock-in C57BL/6-Kit^{W-sh}/Kit^{W-sh} mice engrafted with ET_A receptor-deficient or WT mast cells derived *in vitro* from ET_A^{-/-} or ET_A^{+/+} embryonic stem cells, Maurer et al. found that mast cell activation by ET-1 *via* the ET_A receptor contributed to this effect.⁶⁵

A homology search conducted by Martin Metz revealed that ET-1 was structurally similar to sarafotoxin 6b, one of the major toxins in the venom of the Israeli mole viper (*Atractaspis engaddensis*).⁷⁸ Sarafotoxin 6b can induce activation of cells in the envenomated animals by binding to endothelin receptors.⁷⁹ Metz et al. showed that mast cells not only diminished the toxicity of sarafotoxin 6b and enhanced the survival of mice injected with that peptide, but also did so in mice injected with the whole venom of *A. engaddensis*.¹⁵ Testing the ability of mast cells to influence responses to whole venoms is important, since snakes (and arthropods) don't envenomate their prey with a single toxin but with a complex mixture of toxins that can induce pathology by different mechanisms.⁸⁰ Initial pharmacological studies suggested that chymase was the critical mast cell protease in this setting.⁶⁵ However, later work by our group, employing both pharmacological approaches and shRNA knock down of CPA3 in adoptively-transferred mast cells,¹⁵ as well as elegant studies by Schneider et al., who exploited a mouse they created which expressed only a catalytically inactive CPA3⁸¹, indicated that CPA3 is the key mast cell-derived protease that detoxifies both ET-1 and sarafotoxin 6b. Using mast cell knock-in mice, Metz et al. also provided evidence that mast cells were important in substantially enhancing the innate resistance of mice to honeybee (*Apis mellifera*) venom and to the venoms of two North American pit vipers, the western diamondback rattlesnake (*Crotalus atrox*) and the southern copperhead (*Agkistrodon contortrix contortrix*).¹⁵

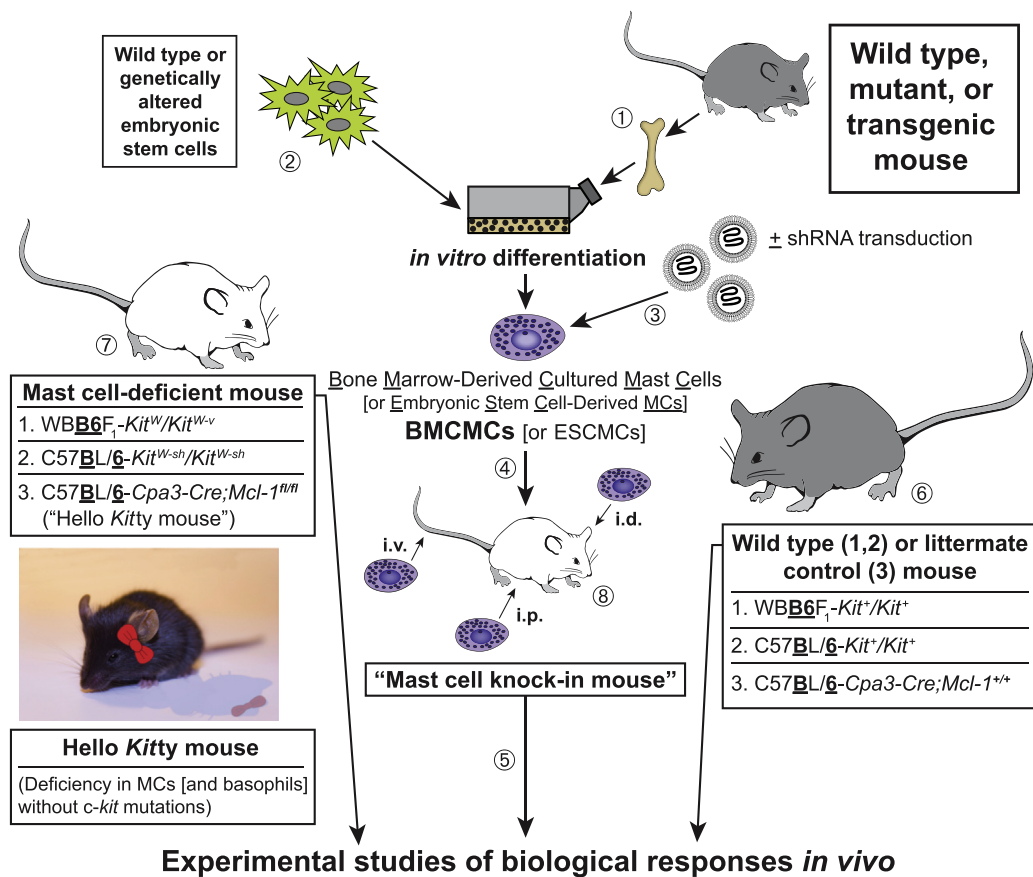


Fig. 2. Making "mast cell knock-in mice". (1) Mast cells can be generated from bone marrow cells (or other hematopoietic cells; e.g., those in the fetal liver) from wild type mice or from mutant or transgenic mice with specific genetic alterations of interest.^{61–63} Alternatively, (2) embryonic stem (ES) cell-derived cultured mast cells (ESCMCs) can be generated from wild type or genetically altered ES cells^{64,65}, or (3) various mast cell populations can be transduced *in vitro* with shRNA to diminish expression of specific genes of interest.^{15,66} (4) Such bone marrow-, ES cell-, [or fetal liver-] derived cultured mast cells, or shRNA-transduced mast cells, can then be transplanted into mast cell-deficient *c-kit* mutant mice, such as $WBB6F_1\text{-}Kit^W/Kit^{W-v}$ mice^{63,67} or $C57BL/6\text{-}Kit^{W-sh}/Kit^{W-sh}$ mice,^{68,69} or into $C57BL/6\text{-}Cpa3\text{-}Cre;Mcl-1^{fl/fl}$ mice⁷⁰ (which we informally refer to as "Hello Kitty mice", which have wild type *c-kit*), to produce mast cell knock-in mice. Note: BMCMCs can be injected into genetically mast cell-deficient mice intravenously (i.v.), intraperitoneally (i.p.), or intradermally (i.d.), or into the joints or meninges, etc., but there is a more limited experience with the engraftment of other types of MCs, such as EMCMCs, than with BMCMCs. (5) A suitable interval is then allowed for engraftment and phenotypic "maturation" of the adoptively-transferred mast cells (the length of this interval can be varied based on the route of mast cell transfer, the anatomical site of interest, the particular biological response being analyzed, etc.). The importance of mast cell function(s) in biological responses can be analyzed by comparison of the responses in the appropriate wild type or littermate control mice (6), the corresponding mutant mast cell-deficient mice (7), and selectively mast cell-engrafted mutant mice (mast cell knock-in mice) (8). The contributions of specific mast cell products (surface structures, signaling molecules, secreted products, and so on) to such biological responses can be analyzed by comparing the features of the responses of interest in mast cell knock-in mice engrafted with wild type mast cells versus mast cells derived from mice or ES cells that lack or express genetically altered forms of such products or that have been transduced with shRNA to silence the specific genes that encode these products. An important part of the analysis of the mast cell knock-in mice used in particular experiments is to assess the numbers and anatomic distribution, and, for certain experiments, aspects of the phenotype, of the adoptively-transferred mast cells, as, depending on the type of *in vitro*-derived mast cells used, the route of administration, and other factors, these may differ from those of the corresponding native populations of mast cells in the corresponding wild type mice.^{14,71,72} [This is a modified version of Fig. 2 in Metz M, Grimbaldston MA, Nakae S, Piliponsky AM, Tsai M, Galli SJ. Mast cells in the promotion and limitation of chronic inflammation. *Immunol Rev* 2007; **217**:304–28 (Ref. 71), reprinted with the permission of the publisher, John Wiley and Sons.]

A project led by Mitsuteru Akahoshi and Chang Ho Song then analyzed whether mast cells might enhance innate resistance to another pair of biologically active peptides, the endogenous mammalian peptide VIP and the structurally similar peptide helodermin (also known as exendin-2), which is one of the toxins present in the venom of the Gila monster (*Heloderma suspectum*).¹⁸ Testing of both mast cell knock-in mice (including $C57BL/6\text{-}Kit^{W-sh}/Kit^{W-sh}$ mice engrafted with WT versus chymase [mMCP4]-deficient mast cells) (Fig. 3A, B) and mice which had mast cells but were genetically deficient in mMCP4⁸² or CPA3⁸¹ or produced a catalytically inactive CPA3⁸¹ (Fig. 3C), showed that mast cells could diminish the toxicity of VIP, helodermin, and the whole venom of *H. suspectum*, and that this was largely or wholly dependent on mast cell-derived mMCP4 rather than CPA3¹⁸. Similar approaches were used to provide evidence that mast cells and mMCP4 can contribute to enhanced innate resistance of mice to the venoms of

two scorpions, one from the old world, the Deathstalker (*Leiurus quiquestriatus hebraeus*), and one from the new world, the Arizona bark scorpion (*Centruroides exilicauda*).¹⁸

It is possible that future work will reveal that mast cell activation can increase, rather than decrease, the toxicity of some venoms. However, our initial evidence indicated that mast cells can increase the innate resistance of mice upon their first exposure to the venoms of 3 species of poisonous snakes, the Gila monster, the honeybee, or two especially dangerous scorpions. Moreover, mast cells contain at least two different proteases, CPA3 and chymase (mMCP4), which permit mast cells to respond, after their activation *via* cognate receptors that can bind either the endogenous or the structurally similar reptile-derived peptides, to high and potentially toxic levels of ET-1 and VIP, respectively, as well as to high levels of the similar peptides contained in the reptile venoms (sarafotoxin 6b in Israeli mole

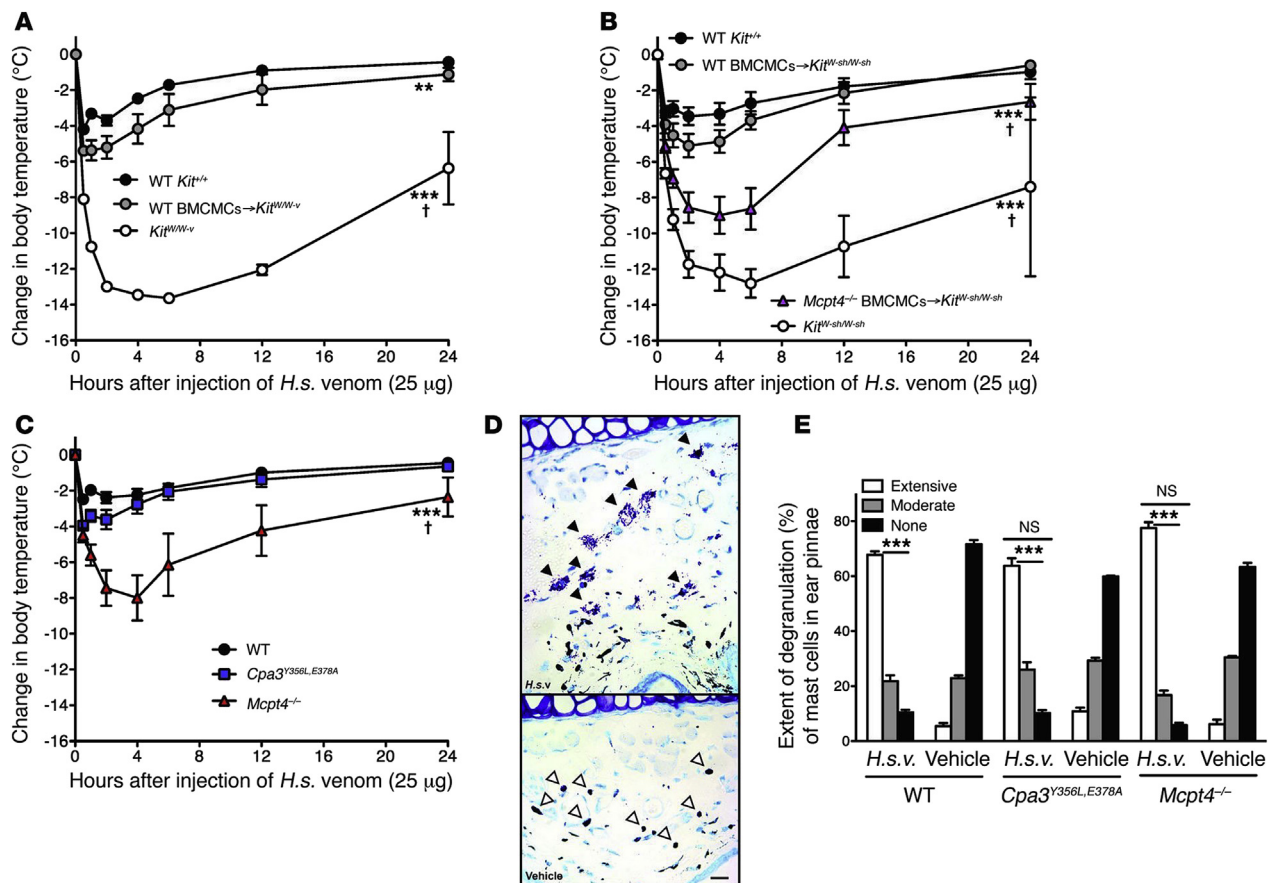


Fig. 3. Mast cells can diminish *Heloderma suspectum* venom (H.s.v.)-induced hypothermia and mortality through MCP4-dependent mechanisms. Changes in rectal temperatures after i.d. injection of H.s.v. (25 µg in 20 µl DMEM solution) into the ear pinnae (one ear pinna of each mouse) of: (A) WT WBB6F1-*Kit*^{+/+}, mast cell-deficient WBB6F1-*Kit*^{W/W-v}, and WT BMCMCs → *Kit*^{W/W-v} mice (i.e., WBB6F1-*Kit*^{W/W-v} mice which had been engrafted, 6–8 weeks before venom challenge, in one ear pinna with 2 million BMCMCs derived from WT WBB6F1-*Kit*^{+/+} mice) (the death rates of *Kit*^{+/+}, WT BMCMCs → *Kit*^{W/W-v}, and *Kit*^{W/W-v} mice within 24 h after H.s.v. injection were 0% [0/21], 7% [1/15, $P = 0.42$ vs. *Kit*^{+/+} mice], and 65% [13/20, $P < 0.0001$ vs. *Kit*^{+/+} mice], respectively); (B) WT C57BL/6-*Kit*^{+/+}, mast cell-deficient C57BL/6-*Kit*^{W-sh/W-sh}, WT BMCMCs → *Kit*^{W-sh/W-sh}, and *Mcpt4*^{-/-} BMCMCs → *Kit*^{W-sh/W-sh} mice (the death rates of *Kit*^{+/+}, WT BMCMCs → *Kit*^{W-sh/W-sh}, *Mcpt4*^{-/-} BMCMCs → *Kit*^{W-sh/W-sh}, and *Kit*^{W-sh/W-sh} mice within 24 h after H.s.v. injection were 5% [1/19], 11% [2/18, $P = 0.48$ vs. *Kit*^{+/+} mice], 43% [6/14, $P = 0.01$ vs. *Kit*^{+/+} mice], and 50% [10/20, $P = 0.006$ vs. *Kit*^{+/+} mice], respectively); or (C) WT C57BL/6-*Kit*^{+/+} mice, C57BL/6-*Cpa3*^{Y356L,E378A} mice (which have a catalytically inactive CPA3) and C57BL/6-*Mcpt4*^{-/-} mice (which are deficient in mMCP4) (the death rates of *Kit*^{+/+}, *Cpa3*^{Y356L,E378A}, and *Mcpt4*^{-/-} mice within 24 h after H.s.v. injection were 7% [1/15], 0% [0/14, $P = 0.52$ vs. *Kit*^{+/+} mice], 40% [6/15, $P = 0.007$ vs. *Kit*^{+/+} mice], respectively). Each figure shows data pooled from at least three independent experiments with each group of mice ($n = 2–5$ mice per group per each individual experiment). ** $P < 0.01$, *** $P < 0.001$ versus WT WBB6F1-*Kit*^{+/+} or WT C57BL/6-*Kit*^{+/+} mice; $P < 0.01–0.001$ versus each other group (A–C). (D) Extensive degranulation of mast cells (some indicated by closed arrowheads) 1 h after i.d. injection of H.s.v. (25 µg in 20 µl DMEM), but not vehicle (DMEM) alone (mast cells without evidence of degranulation are indicated by open arrowheads) in WT C57BL/6 mice (Toluidine blue stain; scale bar: 50 µm). (E) Degranulation of mast cells 60 min after i.d. injection of H.s.v. (25 µg in 20 µl DMEM) or vehicle (DMEM) alone in WT C57BL/6, *Mcpt4*^{-/-}, or *Cpa3*^{Y356L,E378A} mice (injection was into one ear pinna of each mouse). *** $P < 0.001$ versus corresponding vehicle-injected groups; NS = not significant ($P > 0.05$) versus values for WT mice. [This is a reproduction of Fig. 1 from Akahoshi M, Song CH, Piliponsky AM, Metz M, Guzzetta A, Abrink M, Schlenner SM, Feyerabend TB, Rodewald HR, Pejler G, Tsai M, Galli SJ. Mast cell chymase reduces the toxicity of Gila monster venom, scorpion venom, and vasoactive intestinal polypeptide in mice. *J Clin Invest* 2011;121:4180–91 (Ref. 18), reprinted with the permission of the publisher, the American Society for Clinical Investigation.].

viper venom and helodermin in Gila monster venom, respectively) (Fig. 4). By undergoing degranulation and releasing proteases that can inactivate potentially toxic endogenous peptides or peptides in venoms, mast cells can help to restore homeostasis, albeit while also enhancing features of the ensuing local and perhaps systemic inflammatory responses.

Depending on the mammalian species, mast cells can contain several tryptases and chymases of distinct substrate specificity, as well as CPA3^{13,72,83}. This raises the possibility that one of the reasons that the mast cells of various species contain several different proteases in their cytoplasmic granules is so that these cells, which are positioned in large numbers in the skin, the most common site of envenomation, are equipped to release a panel of proteases with the potential to degrade a variety of structurally distinct toxic compounds contained in animal venoms. Mast cells might also contribute to innate resistance to venoms in other ways, such as by increasing local vascular permeability and thereby favoring the

interstitial access of circulating molecules that can antagonize the effects of venom proteases⁸⁴ and other toxins.

IgE can contribute to host defense against arthropod and reptile venoms

Many animals and some humans experience multiple episodes of envenomation by arthropods such as bees, wasps, and scorpions, or by various reptiles. Such envenomation not only provokes an innate inflammatory response and pathology related to the biological activities of the venoms' toxins,^{85–87} but also can induce allergic sensitization associated with the development of specific IgE antibodies.^{88–93} In some unfortunate people, such IgE responses to venoms put these individuals at risk to develop potentially fatal episodes of anaphylaxis.^{3,7,23,93} But recent findings suggest, in accord with Profet's "toxin hypothesis of allergy", that

Whole animal venom (containing many toxins)	Israeli mole viper (See Ref. 15) 	Gila monster (See Ref. 18) 
Exogenous toxin in animal venom	Sarafotoxin-6b (See Refs. 15 & 81)	Helodermin (See Ref. 18)
(similar peptides)		
Potentially toxic endogenous peptide	ET-1 (endothelin-1) (See Refs. 15, 65 & 81)	VIP (vasoactive intestinal polypeptide) (See Ref. 18)
Mast cell product that degrades peptides and enhances survival after injection of venom	Carboxypeptidase A3 (mCPA3)	Mast cell protease 4 (mMCP4)

Fig. 4. Mast cells can enhance innate resistance to high levels of endogenous peptides and structurally similar peptides in reptile venoms. Mast cell cytoplasmic granules contain proteases such as carboxypeptidase A3 (CPA3 [mCPA3 = mouse CPA3]) and mast cell protease 4 (MCP4 [mMCP4 = mouse MCP4]) that, upon secretion by activated mast cells, can degrade certain endogenous peptides, such as endothelin-1 (ET-1) and vasoactive intestinal polypeptide (VIP), respectively, as well as structurally similar peptides contained in the venoms of poisonous reptiles, such as sarafotoxin 6b in the venom of the Israeli mole viper (*Atractaspis engaddensis*) and helodermin in the venom of the Gila monster (*Heloderma suspectum*). The ability of mast cells to be activated to degranulate by components of venoms such as these, which can act at the same receptors which recognize the corresponding structurally similar endogenous peptides, permits mast cells to release proteases that can reduce the toxicity of these peptides and which help to enhance the survival of mice injected with the whole venoms of these reptiles, that contain many toxins in addition to sarafotoxin 6b and helodermin. This mechanism may also permit mast cells to restore homeostasis in settings associated with markedly increased levels of the endogenous peptides. [This is a reproduction of Fig. 4 from Galli SJ. The 2014 Rous-Whipple Award Lecture. The mast cell-IgE paradox: From homeostasis to anaphylaxis. *Am J Pathol*, in press (ref. 42), reprinted with the permission of the publisher, Elsevier for the American Society for Investigative Pathology. The photograph of the Israeli mole viper is by Amikam Shooob, courtesy of Elazar Kochva, Tel Aviv University, and the photograph of the Gila monster is from reptiles4all and is used under license from Shutterstock.com].

this same “allergic” mechanism – involving IgE and mast cells – also can enhance host resistance to venoms.

Honeybee (*A. mellifera*) venom consists of a mixture of cytolytic peptides (e.g., melittin), enzymes (e.g., phospholipase A₂ [PLA₂; considered the main allergen in bee venom]), hyaluronidase, neurotoxins and bioactive amines⁸⁵ and accounts for a large fraction of venom allergies in humans.⁹³ The venom of the Russell's viper (*Daboia russelii*), one of the most dangerous snakes in the Indian subcontinent,⁹⁴ is a complex mixture of growth factors and enzymes with pro-coagulant and neurotoxic activities.⁸⁷ We found that, in mice, IgE-associated type 2 immune responses against honeybee venom or Russell's viper venom were able to increase significantly host resistance to challenge with potentially lethal doses of those venoms.⁹⁵

This was unexpected because both IgE and IgG₁ antibodies produced during type 2 immune responses can orchestrate anaphylaxis and other allergic reactions in mice^{7,23,96,97} and because type 2 immune responses against venoms (that include the development of anti-venom IgG₁ [in mice] and IgE antibodies) are classically thought to *exacerbate* the outcome of subsequent venom exposure.^{89–93,98} By contrast, IgG class antibodies raised against animal venoms (or their F(ab')₂ fragments), are used to treat envenomated humans or animals.⁹⁹

So it was important to identify which antibodies contributed to the enhanced resistance to honeybee venom observed in mice with

type 2 immune responses to that venom. Our evidence showed that IgE antibodies were the critical elements of the acquired host resistance to honeybee or Russell's viper venom. We found: 1) that most or all of the acquired resistance induced in mice by a single exposure to honeybee venom was transferable to naïve mice with only 250 µL of serum from honeybee venom-immunized mice; 2) that when such “immune serum” was depleted of IgE either by adding a neutralizing antibody to IgE^{35,100} or by heating (56 °C, 1 h, which eliminates the ability of IgE to bind to FcεRI and induce passive cutaneous anaphylaxis¹⁰¹ while the function of other antibody classes, including IgG₁, is not affected¹⁰²), the immune serum's ability to transfer enhanced resistance to naïve mice was essentially lost; and 3) that such “immune serum” failed to transfer enhanced venom resistance to mice lacking either the IgE antibody-binding α chain of the FcεRI or the γ chain of FcεRI that is necessary for signaling initiated by aggregation of the receptor.^{5,6}

We also found: 1) that genetically IgE-deficient mice⁹⁶ could not develop acquired immunity to honeybee venom, even though they developed a robust IgG₁ antibody response to the venom; 2) that “immune serum” from WT mice could passively transfer enhanced resistance to honeybee venom to naïve IgE-deficient mice, unless such “immune serum” was first treated to neutralize IgE or impair its ability to bind to FcεRI, and 3) that naïve genetically mast cell-deficient C57BL/6-Kit^{W-sh/Kit^{W-sh}} or C57BL/6-Cpa3-Cre⁺-Mcl-1^{fl/fl} mice which received immune serum from honeybee venom-immunized C57BL/6 WT mice actually exhibited worse survival after challenge with a high dose of honeybee venom than did mast cell-deficient mice which had received serum from PBS mock-immunized C57BL/6 WT mice.⁹⁵ The latter finding suggested that mast cells can contribute to IgE-mediated acquired resistance to honeybee venom, as well as enhance the innate resistance of mice to a first exposure to that venom.¹⁵ Independently of our work, Palm et al. showed that mice immunized with the major allergen contained in honeybee venom, bee venom phospholipase A₂ (bvPLA₂), exhibited enhanced resistance to the ability of bvPLA₂ to induce hypothermia upon its injection into mice, and provided evidence that this enhanced immunity required B cells and was diminished significantly in mice which lacked the IgE-binding α chain of the FcεRI¹⁰³. Taken together, these two initial studies^{96,103} support the hypothesis that one physiological function of IgE is to protect the host against noxious substances.

Subsequently, we found that the acquired enhanced resistance to Russell's viper venom (RVV) which we observed in mice that had developed type 2 immune responses to that venom⁹⁵ also was highly dependent on IgE (Fig. 5) and FcεRI (see Fig. 4, A–E in Ref. 104), and could be effectively transferred by immune serum into normal mice (see Fig. 3, F–J in Ref. 104) but not into C57BL/6-Cpa3-Cre;Mcl-1^{fl/fl} mice which were genetically markedly deficient in mast cells and which also had diminished numbers of basophils (see Fig. 4, G and H in Ref. 104). Notably, two different types of genetically mast cell-deficient mice also exhibited significantly diminished *innate* resistance to the toxicity and lethality of RVV (Fig. 6A–C, E, F), supporting Higginbotham's hypothesis that mast cells can contribute to enhanced innate resistance to this venom.⁷⁶ Compared to the corresponding mast cell-sufficient mice, such naïve mast cell-deficient mice also exhibited many fewer attempts to scratch sites of RVV injection (Fig. 6D, G). The latter finding supports the idea proposed both by Stebbings⁴⁰ and Profet³⁹ that elements of allergic responses, in this case, mast cells, can confer benefit to hosts experiencing attacks by arthropods⁴⁰ or other sources of toxins³⁹ by altering the host's behavior in ways that would help to eliminate, or at least permit the host to become aware of, the threat.

As noted above, Palm et al. reported that immunization of mice with honeybee venom-derived bvPLA₂, which represents

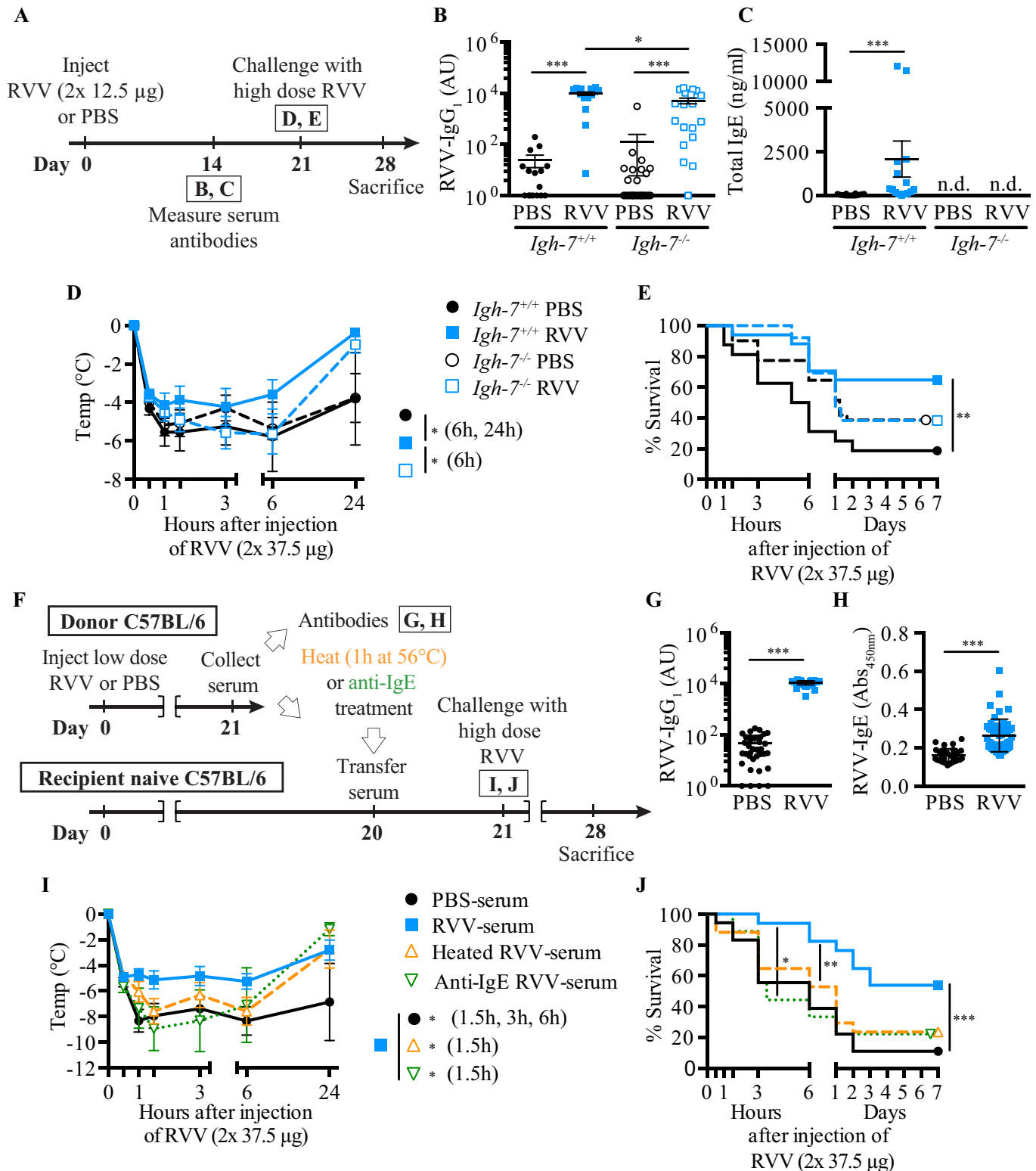


Fig. 5. Evidence that IgE antibodies contribute to acquired enhanced resistance to the toxicity and lethality of Russell's viper venom. A. Outline of experiments with IgE-deficient (*Igh-7^{-/-}*) and control (*Igh-7^{+/+}*) C57BL/6 mice (B–E). B, C. Serum RVV-specific IgG₁ (B) and total IgE (C). D, E. Body temperature (D) and survival (E). F. Outline of serum transfer experiments in C57BL/6 mice (G–J). G, H. Serum RVV-specific IgG₁ (G) and RVV-specific IgE (H). I, J. Body temperature (I) and survival (J). Data were pooled from 3 to 4 experiments (n = 9–25/group). P values: Mann–Whitney test (B, C, G, H), Student's *t* test (D, I) and Mantel–Cox test (E, J). [This is a reproduction of Fig. 3 from Starkl P, Marichal T, Gaudenzio N, Reber LL, Sibillano R, Tsai M, Galli SJ. IgE antibodies, FcεR1α and IgE-mediated local anaphylaxis can limit snake venom toxicity. *J Allergy Clin Immunol*. In press (Ref. 104), reprinted with the permission of the publisher, Elsevier].

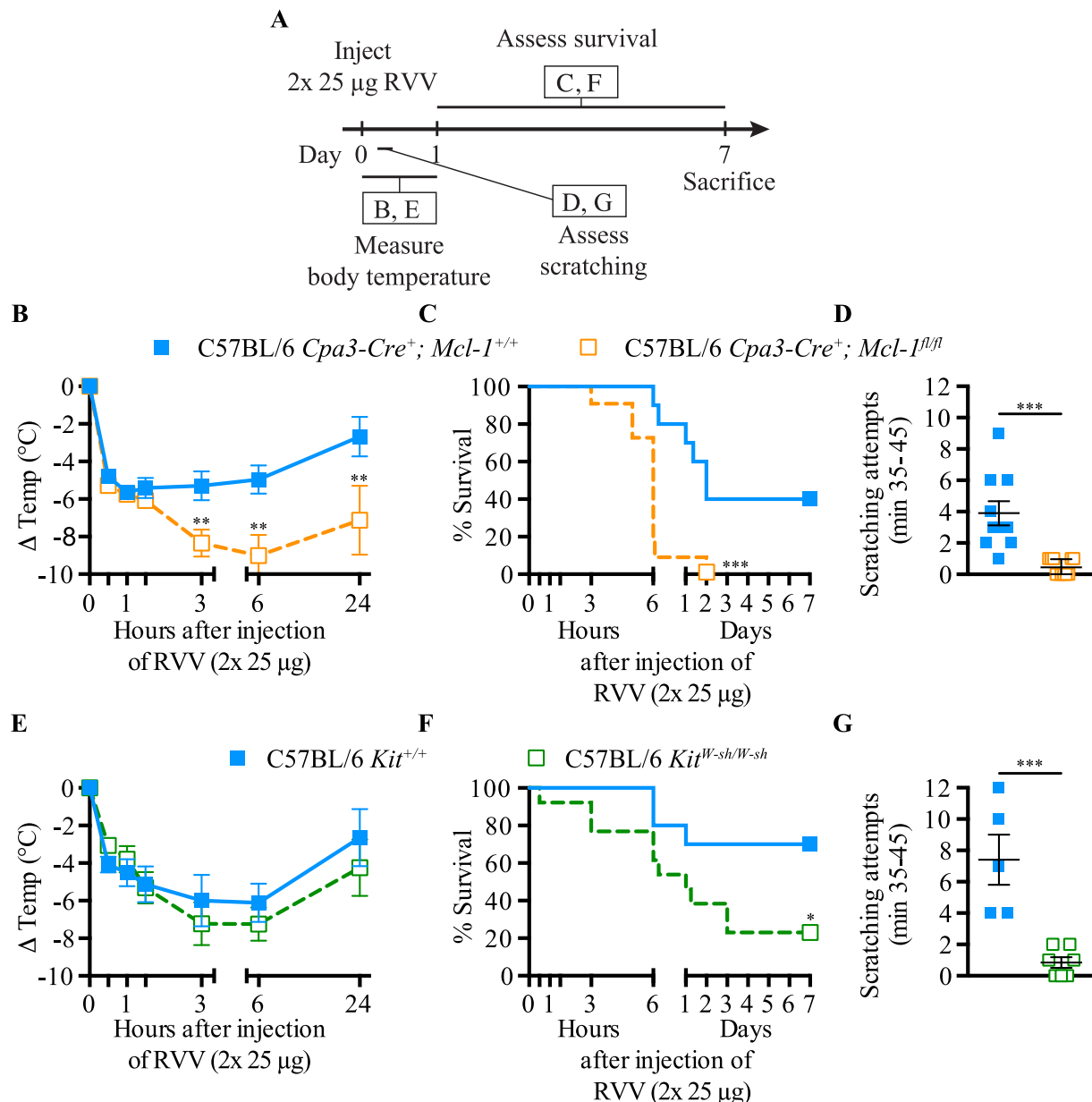


Fig. 6. Evidence that mast cells contribute to innate resistance to the toxicity and lethality of Russell's viper venom, as well as to behavioral responses to envenomation. **A.** Experimental outline. **B** and **E**, body temperature; **C** and **F**, survival; **D** and **G**, scratching attempts, of mast cell-deficient *Cpa3-Cre⁺; Mcl-I^{fl/fl}* (**B–D**) and *Kit^{W-sh/W-sh}* (**E–G**) mice and corresponding control mice after RVV injection. *P* values: (**B**, **D**, **E**, **G**) Student's *t* test; (**C**, **F**) Mantel–Cox test. Data were pooled from 2 to 4 experiments (*n* = 5–21/group). [This is a reproduction of Fig. 2 of Starkl P, Marichal T, Gaudenzio N, Reber LL, Sibillano R, Tsai M, Galli SJ. IgE antibodies, FcεRIα and IgE-mediated local anaphylaxis can limit snake venom toxicity. *J Allergy Clin Immunol*. In press (Ref. 104), reprinted with the permission of the publisher, Elsevier.].

approximately 10% of the dry weight of whole BV¹⁰⁵ can reduce the toxicity-related hypothermia induced by subsequent challenge with a high dose of the same allergen in an antibody- and FcεRIα-dependent manner.¹⁰³ However, it was not clear whether an IgE response to a single constituent of an animal venom would be able to enhance resistance to the entire group of toxins contained in that venom. To investigate this, we passively sensitized WT mice locally against DNP-HSA by s.c. injections of anti-DNP IgE¹⁰⁶ (or with anti-DNP IgG₁ or IgG_{2b} as controls), or mock-sensitized the mice with saline, then challenged the animals s.c. at the same site 24 h later by injecting a mixture of RVV and DNP-HSA (Fig. 7A). We used amounts of anti-DNP IgE and DNP-HSA which were able to induce a local increase in vascular permeability at the DNP-HSA injection site without resulting in systemic hypothermia, and showed that

the amount of DNP-HSA used did not by itself influence the toxicity of RVV (see Fig. E5 in the Online Repository of Ref. 104).

We found that pre-sensitization with anti-DNP IgE significantly increased the resistance of C57BL/6 (Fig. 7B, C) or BALB/c (see Fig. E5, H–I in the online repository of Ref. 104) mice to challenge with a potentially lethal amount of RVV admixed with DNP-HSA.¹⁰⁴ However, pre-sensitization of C57BL/6 mice with anti-DNP IgG₁ or IgG_{2b}, DNP-specific IgG isotypes with the capacity to activate effector cells via Fcγ receptors¹⁰⁷, not only failed to increase protection but also resulted in increased hypothermia at early time points compared to vehicle-treated or IgE-sensitized mice (Fig. 7B).¹⁰⁴ These findings show that local tissue responses mediated by IgE and antigen can enhance host resistance against RVV even when that antigen is not a native constituent of the venom,

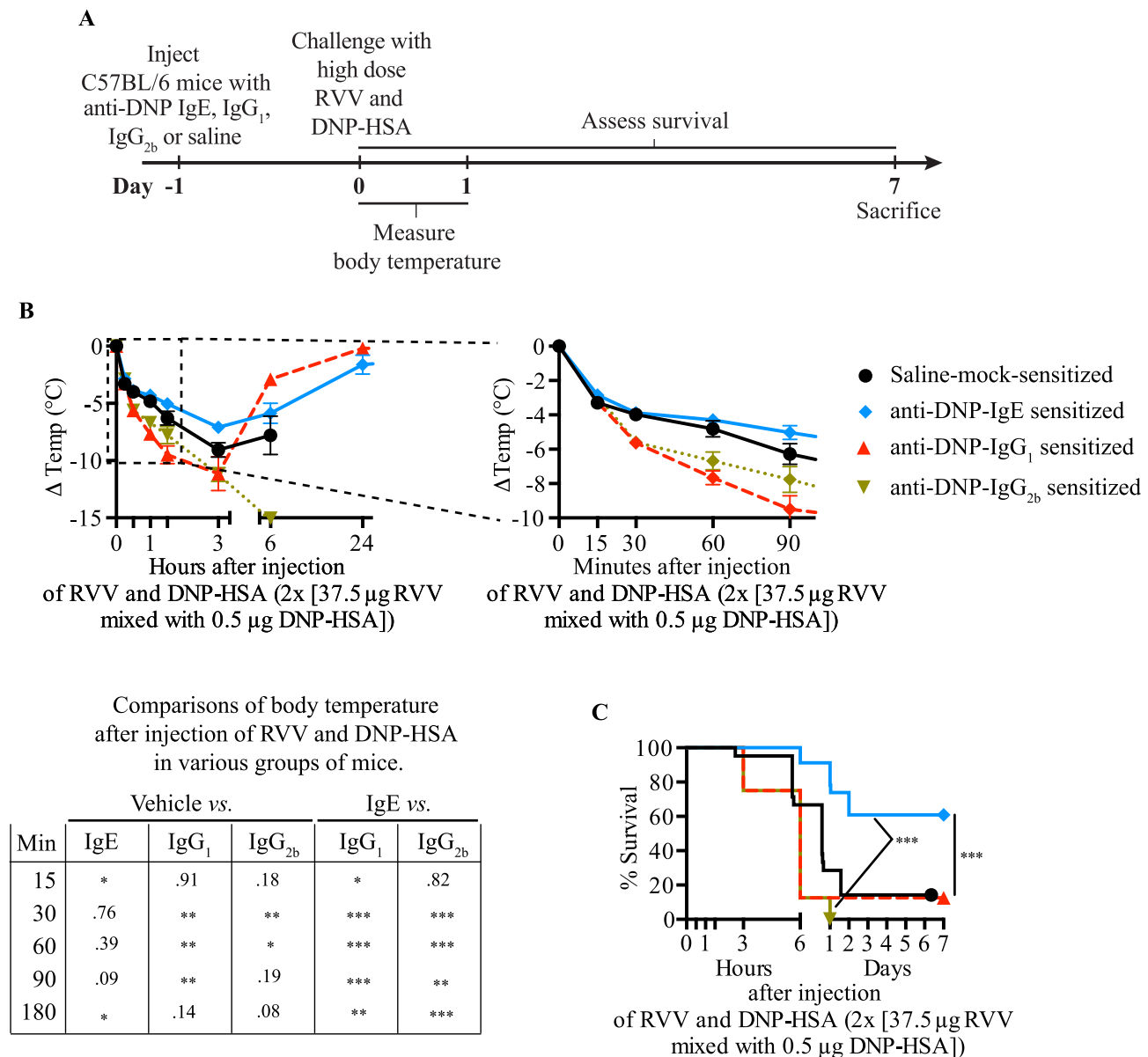


Fig. 7. IgE-dependent local mast cell activation induced by activation with a single antigen can enhance resistance to the lethality of Russell's viper venom. **A.** Experimental outline. **B, C.** Body temperature (B) and survival (C) of C57BL/6 mice treated with 3 s.c. injections of saline alone or containing 50 ng anti-DNP IgE, IgG₁ or IgG_{2b} antibody and challenged 18 h later with 2 s.c. injections, each containing 37.5 μ g RVV and 0.5 μ g DNP-HSA. Data were pooled from 2 to 5 independent experiments ($n = 10-25$ /group). P values: Student's t test (B); Mantel-Cox test (C). [This is a reproduction of Fig. 5 from Starkl P, Marichal T, Gaudenzio N, Reber LL, Sibillano R, Tsai M, Galli SJ. IgE antibodies, Fc ϵ RI α and IgE-mediated local anaphylaxis can limit snake venom toxicity. *J Allergy Clin Immunol*. In press (Ref. 104), reprinted with the permission of the publisher, Elsevier.].

and are consistent with the general idea that the host needs only to generate an IgE response against a limited number of the components of a complex venom (perhaps as few as one component) in order to manifest enhanced acquired resistance to that venom.

Conclusions

Tissue resident cells with morphological, biochemical, and functional properties of mammalian mast cells, and which can produce histamine, heparin and serine proteases, are present in tunicates, whose ancestors appeared in evolution before the development of adaptive immunity.^{108,109} Such tunicates also have been reported to have cells resembling basophils.¹¹⁰ After the appearance of acquired immunity and the development of antibodies, these ancient hematopoietic lineages acquired the ability to

bind immunoglobulins such as IgE (in mammals) to their surface. This allowed such tissue-resident cells to become "immunologically primed" or "sensitized" to undergo activation for mediator release upon encountering relatively small amounts of the antigen identified by their surface-bound IgE antibodies. The most extreme example of an IgE-associated immune response resulting in the activation of mast cells (and basophils) is fatal anaphylaxis, in which the rapid, systemic and extensive release of mediators stored in these Fc ϵ RI-bearing effector cells results in a catastrophic and quickly lethal outcome.

Observational and epidemiological studies in humans, as well as studies in experimental animals (including those employing mice genetically deficient in mast cells, basophils or IgE), strongly suggest that one beneficial role of IgE, mast cells and basophils is to help to defend the host against ectoparasites such as ticks, and to diminish

the numbers of parasites and burden of disease in mammals infected with certain helminths. However, in addition to parasites, vertebrates also have been subjected to evolutionary pressure through millions of years of co-evolution with venomous arthropods, reptiles, and other species. Evidence in mice indicates that mast cells can enhance innate resistance of mice to 4 species of poisonous snakes, the Gila monster, 2 species of scorpions, and the honeybee, and that mast cell proteases (specifically, CPA3 and the chymase MCP4) can contribute to such mast cell-dependent innate defenses by degrading toxins present in some of these venoms. Moreover, type 2 immune responses induced by a single exposure to honeybee venom or Russell's viper venom, which "arm" mast cells with IgE antibodies that bear specificity for components of those venoms, can significantly increase the survival of such mice to challenge with doses of the venoms which would be lethal in naïve mice.⁹⁵

This evidence supports the notion^{39–41} that key elements of "allergic reactivity", including mast cells and IgE, indeed can importantly enhance innate and acquired host resistance to venoms. Yet much work remains to be done to answer several related, but unresolved, questions. These include: 1) in addition to releasing proteases, are there other mechanisms by which mast cells can contribute to enhanced resistance to venoms during innate or acquired immune responses [e.g., *ex vivo* studies indicate that mast cell-derived heparin, that is highly anionic, can bind and thereby reduce the toxicity of cationic toxins in Russell's viper venom⁷⁶]; 2) in what ways do venoms induce Th2 cell and IgE responses (for honeybee venom, this appears to involve a pathway by which products of *bvPLA₂* acting on host lipid membranes induce IL-33 production, which in turn can activate ILC2 cells to release cytokines that drive IgE production¹⁰³); 3) during vertebrate evolution, what has been the relative importance of exposure to ectoparasites (and the pathogens for which they serve as vectors), infection with helminths and other parasites, and interactions with venomous animals in shaping the features, function and immunological roles of mast cells, basophils, and IgE?; 4) Given that mast cells and basophils cooperate to enhance acquired resistance to the feeding of certain ticks, and that the hematophagous fluids produced by tick salivary glands can contain peptides similar to those in venoms,¹¹¹ is there a role for basophils in enhancing resistance to some venoms?; and 5) why do some subjects develop such severe IgE-dependent reactivity to venom that they are at risk for fatal anaphylaxis (an outcome far from a "protective" immune response).

Our initial findings indicate that the propensity to develop protective vs. potentially harmful type 2 immune responses to venoms, at least in mice, can depend on the genetic background of the animal, the type and amount of venom to which the animal is exposed, and/or the frequency of such venom exposures.¹⁰⁴ But this is only the beginning of addressing this important issue. In considering this question, it should be noted that many people who develop type 2 immune responses to BV do not exhibit anaphylactic reactivity despite having venom-specific IgE antibodies.¹¹² Also, there is abundant evidence that Th2 cell-mediated responses are subject to immune regulation which can diminish pathology related to IgE-dependent reactivity to the inducing antigen, including honeybee venom.^{113–115} One might speculate that such immune regulation of type 2 immune responses ideally would reduce the pathology associated with these responses while preserving their ability to confer enhanced protection when the eliciting antigen is a toxin.

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Conflict of interest

The authors have no conflict of interest to declare.

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