ROUS-WHIPPLE AWARD LECTURE

The Mast Cell-IgE Paradox

From Homeostasis to Anaphylaxis

Stephen J. Galli

From the Departments of Pathology and Microbiology and Immunology and the Sean N. Parker Center for Allergy Research, Stanford University School of Medicine, Stanford, California

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Address correspondence to
Stephen J. Galli, M.D., Department
of Pathology, Stanford
University School of Medicine,
L-235, 300 Pasteur Dr., Stan-
ford, CA 94305-5324. E-mail:
sgalli@stanford.edu.

Mast cells and IgE are so inextricably linked to the pathology of allergic disorders, including fatal anaphylaxis, that it can be difficult to think of them in other contexts. Surely, we do not have mast cells and IgE so that we can eat a peanut and die! It is thought that mast cells and IgE and basophils (circulating granulocytes, whose functions partially overlap with those of mast cells) can contribute to host defense as components of adaptive T helper cell type 2 immune responses to helminths, ticks, and certain other parasites. Accordingly, it was suggested that allergies are misdirected type 2 immune responses in which IgE antibodies are produced against any of a broad variety of apparently harmless antigens. However, components of animal venoms also can sensitize individuals to develop severe IgE-associated allergic reactions, including fatal anaphylaxis, on subsequent venom exposure. Here, I describe evidence that mast cells can enhance innate host resistance to reptile or arthropod venoms during responses to an initial exposure to such venoms and that acquired type 2 immune responses, IgE antibodies, the high-affinity IgE receptor FcεRI, and mast cells can contribute toward acquired resistance in mice to the lethal effects of honeybee or Russell's viper venom. These findings support the hypothesis that mast cells and IgE can help protect the host against noxious substances. (*Am J Pathol* 2016, 186: 212–224; <http://dx.doi.org/10.1016/j.ajpath.2015.07.025>)

Mast Cells, Basophils, and IgE in the Pathology of Allergic Disorders

Allergies, which afflict 20% to 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 that 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 orchestrates antigen-specific effector function by binding to the high-affinity receptor for IgE (FcεRI)^{5,6} that is expressed on the surface of mast cells (that reside in most vascularized tissues in mammals and other vertebrates) and basophilic granulocytes (basophils ordinarily circulate in low numbers in the blood but can be recruited to sites of inflammation).^{3,5–10}

When mast cell- or basophil-bound IgE recognizes antigens that are at least bivalent, aggregation of the FcεRI rapidly occurs, initiating a complex signaling cascade that results in the release, by such activated mast cells and basophils, of a wide spectrum of mediators that have diverse biological effects.^{5,6,8–11} These mediators include molecules

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stored in the cytoplasmic granules of the cells (ready for immediate release), such as in mast cells, histamine, heparin, and other proteoglycans; proteases such as carboxypeptidase A3, tryptases, and chymases; some cytokines that can be contained in the granules; products of arachidonic acid metabolism via the cyclo-oxidase or lipoxygenase pathways (eg, prostaglandins and cysteinyl leukotrienes); and a diverse group of cytokines, chemokines, and growth factors that are transcriptionally up-regulated and secreted as a result of FcεRI-dependent cell activation.^{3,5–7,12,13} Basophils activated via FcεRI aggregation can release a group of mediators partially overlapping with those of mast cells, but they contain, for example, much lower amounts of proteases and, compared with mast cells, appear to represent a source of fewer cytokines and chemokines.^{8–10}

Innate Mechanisms of Mast Cell Activation

It is now well established that at least some populations of mast cells also can be activated by many stimuli via innate mechanisms that operate independent of IgE, including products of complement activation (eg, C3a, C5a), products of pathogens (eg, lipopolysaccharide and other pathogen-associated molecular patterns), 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], 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} Moreover, 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, but less potently elicit release of lipid mediators or cytokines than would the same cells activated via the FcεRI.^{14,20,21} By contrast, for at least some mast cell populations, pathogen-associated molecular patterns are more effective in eliciting release of cytokines and chemokines than granule-stored mediators.^{16,17} When one also considers that, during innate or adaptive immune responses, mast cells (or basophils) may encounter several different stimuli of activation, simultaneously or sequentially, one appreciates the difficulty of predicting the nature, amount, and net effects of mast cell- or basophil-derived mediators, in particular biological responses.

Hypotheses about the Beneficial Functions of Mast Cells, Basophils, and IgE

It has long been accepted that mast cell and basophil activation can contribute importantly to the pathology

associated with allergic disorders, including potentially fatal anaphylaxis^{3,22,23}; however, the evolutionary advantage conferred by IgE, mast cells, and basophils remains unknown. A major hypothesis about the potential beneficial functions of such allergic effectors has focused on host responses to parasites. During infections with helminths and certain other parasites, IgE-associated Th2-cell-mediated responses are thought to contribute to host defense.^{4,24–26}

Although widely accepted, proving that IgE (or mast cells or basophils) dramatically influences the survival of parasite-infected animals has been challenging. To be sure, abnormalities in host responses to certain parasites were 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. Moreover, there are even suggestions that in certain settings IgE or mast cells may have effects during host responses to parasites (eg, effects that directly or indirectly result in increased parasite egg production) that may favor the parasite rather than the host.^{34–36}

When viewed from the perspective that vertebrates have been co-evolving with parasites for millions of years and that the parasites' objectives may better be served by a viable rather than dead host, it is not surprising that, depending on the parasites and the particular setting, effector mechanisms such as IgE, mast cells, and basophils may be exploited by the parasites to their own advantage. For example, 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 may alter local blood flow and vascular permeability in ways that enhance the parasite's ability to derive nutrition from the host.

Unlike parasites, most allergens do not represent a direct threat to the nonsensitized host. Accordingly, type 2 immune responses that result in antigen-specific IgE antibodies to such allergens are widely considered to be misdirected and maladaptive immune responses.^{37,38} However, a radically different conclusion was drawn by Margie Profet,³⁹ who in 1991 suggested that the common feature of most allergens is their origin from sources (such as seafood, nuts, or venoms) which sometimes (eg, foods) or always (eg, venoms) contain toxins. She also proposed that acute allergic reactions, manifested as immediately occurring symptoms in response to allergen exposure, such as sneezing, coughing, vomiting, or diarrhea, evolved as a defense mechanism to allow the sensitized host to respond immediately to and to expel, neutralize, and/or avoid noxious substances that might be indicative of potentially life-threatening situations.³⁹ James Stebbings, Jr.⁴⁰ earlier 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.”^{pp.40} However, until recently,⁴¹ Profet's toxin hypothesis, was largely ignored by the scientific

community; Stebbings' article was even more neglected. According to Google Scholar on March 6, 2015, the reviews of Profet³⁹ and Stebbings⁴⁰ were cited 77 and 24 times, respectively.

In the remainder of this article, I describe how my colleagues and I, by pursuing our efforts to understand the functions of basophils and mast cells *in vivo* and building on important work by many other groups, inadvertently uncovered evidence that supports the hypotheses of Stebbings⁴⁰ and Profet³⁹ that key effector elements of allergic diseases, namely basophils, mast cells, and IgE antibodies, also can contribute to defense against arthropods and animal venoms.

Evidence for a Beneficial Role of Basophils in Acquired Immunity to the Feeding of Ixodid Ticks

During my postdoctoral training with Hal Dvorak, I first focused on projects in basophil biology, including work with Anne Galli and Ann and Hal Dvorak which demonstrated that guinea pig basophils could synthesize histamine from histidine.⁴² This sparked a personal fascination with these cells and, in particular, with the problem of understanding their physiologic functions. My colleagues and I developed a rabbit anti-guinea pig basophil antiserum, in the hope that this would permit us to discern some of the roles of these cells in health and disease.⁴³ Notably, *i.v.* administration of the antiserum markedly reduced numbers of blood basophils *in vivo* without reducing the numbers of blood eosinophils or the total white blood cell count or changing the numbers of intradermal mast cells. However, the anti-basophil serum caused a reduction in skin mast cell numbers if injected intradermally, suggesting that the antiserum targeted an antigen shared by basophils and mast cells. When we administered this antiserum *i.v.* to guinea pigs sensitized to express cutaneous basophil hypersensitivity, an antigen-induced delayed-onset erythematous skin reaction that contains large numbers of infiltrating basophils,⁴⁴ we uncovered an unexpected result. We anticipated we would observe milder cutaneous basophil hypersensitivity reactions in the anti-basophil serum-treated guinea pigs than in guinea pigs treated with normal rabbit serum; however, we observed the opposite effect. Although the numbers of basophils (but not mononuclear cells, eosinophils, neutrophils, or mast cells) at cutaneous basophil hypersensitivity reaction sites were markedly diminished in guinea pigs treated with anti-basophil serum versus normal rabbit serum, the reactions were significantly more erythematous in the guinea pigs with reduced numbers of basophils.⁴³ Although other possible explanations for this finding were not formally excluded, our observations raised the possibility that basophils might have functions that restrain the development of erythema in this specific setting.

I then had the good fortune to collaborate with Stephen Brown, Gerald Gleich, and Phil Askenase, on efforts to understand whether basophils or eosinophils contributed to adaptive immune response that can diminish the feeding success of larval ticks. In this model, guinea pigs were first subjected to a single exposure to the feeding of larval *Amblyomma americanum* ticks. *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.⁴⁵ 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 nonprimate meat and meat products, sensitizing such individuals to develop a delayed form of urticaria or anaphylaxis after consumption of such meats or meat products⁴⁶ or in response to treatment with a therapeutic antibody that contains this carbohydrate.⁴⁷

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 naive guinea pigs.⁴⁸ In addition, tick feeding sites in sensitized guinea pigs contain large numbers of basophils and eosinophils.⁴⁹ But were these cells helping to enhance host resistance to tick feeding? We found that the treatment of tick-sensitized guinea pigs with the rabbit anti-basophil serum not only markedly diminished basophils in the bone marrow, blood, and skin at sites of tick feeding of the sensitized guinea pigs (and resulted in diminished numbers of eosinophils at such feeding sites) but also essentially ablated the acquired resistance to tick feeding conferred by sensitization⁵⁰ (Figure 1). We also found that rabbit anti-eosinophil serum had a lesser but still statistically significant effect on tick immunity, whereas normal rabbit serum had no effect (Figure 1).⁵⁰

To our knowledge, this was the first experimental evidence (beyond correlative and observational studies to demonstrate the presence of basophils at tick feeding sites) to indicate that basophils might be an 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 Yukihiro Kitamura and colleagues⁵¹ used mast cell knockin mice and Hajime Karasuyama and colleagues⁵² used mice genetically deficient in basophils to show that mast cells and basophils, as well as IgE, can contribute to acquired immunity to the feeding of *Haemaphysalis longicornis* ticks in mice. By contrast, basophils were suggested to have a more important role than mast cells in acquired

Figure 1 Effects of treatment with ABS, AES, or NRS on feeding success of larval *Amblyomma americanum* ticks in guinea pigs. One hundred larval *A. americanum* ticks were placed on the flanks of nonsensitized (naive) guinea pigs or hosts sensitized 26 days earlier by a primary infestation of *A. americanum*. **A–E:** The naive 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 in Brown et al.⁵⁰ The number (Tick Yield) and weight (Tick Weight) of engorged ticks was determined at 90 hours of infestation. ABS completely ablated immunity; AES partially impaired resistance; NRS had no effect. Data are expressed as means $\pm$ SEM. $n = 3$ 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. ABS, anti-basophil serum; AES, anti-eosinophil serum; NRS, normal rabbit serum. Adapted and modified from Brown et al.⁵⁰ with permission from the American Association of Immunologists, Inc.

resistance to the feeding of larval *Dermacentor variabilis* ticks in mice.⁵³

Development of Methods to Generate Large Numbers of Immature Mast Cells *in Vitro* and to Produce Mast Cell Knockin Mice

A main factor in shifting my research focus from basophils to mast cells was the seminal set of observations of Yuki-hiko Kitamura et al,⁵⁴ who discovered that (WB/Re-W/+ X C57BL/6-Wⁿ/+) F_1 -W/Wⁿ mice (now known as WBB6F₁-Kit^W/Kit^{W-n} mice, because W later was shown to encode *c-kit*^{55,56}) not only had a moderate macrocytic anemia (a phenotype known for decades) but were profoundly deficient in tissue mast cells. Notably, mast cells could develop in WBB6F₁-W/Wⁿ mice engrafted with bone marrow cells from the wild-type littermate WBB6F₁-^{+/+} mice.⁵⁴ However, the recipient W/Wⁿ mice also were cured of their anemia,⁵⁴ as was initially shown by Elizabeth Russell.⁵⁷ Moreover, eventually, nonirradiated WBB6F₁-W/Wⁿ mice engrafted with sufficient (eg, 1×10^7) WBB6F₁-^{+/+} or other genetically compatible wild-type whole bone marrow cells also underwent virtually complete replacement of multiple other hematopoietic lineages (including granulocytes and lymphocytes) with cells of donor origin.^{58–60}

Because the transfer of wild-type bone marrow cells into W/Wⁿ mice did not result in the selective adoptive transfer of mast cells, due to the presence of hematopoietic stem and progenitor cell populations in such bone marrow cells, I wondered whether a selective repair of the mast cell deficiency of W/Wⁿ mice might be achieved by transferring lineage-committed mast cells to the mice instead of whole

bone marrow cells. Working with Gary Nabel when he was a graduate student in Harvey Cantor's laboratory, my colleagues and I developed approaches to generate *in vitro* from mouse hematopoietic progenitor cells large populations of immature mast cells, so characterized on the basis of their morphologic, ultrastructural, biochemical, and functional features.^{61,62} Notably, Ann Dvorak was certain that these were mast cells based solely on their ultrastructural features, but we identified several other characteristics of mast cells in these *in vitro*-derived cells, including their ability to synthesize histamine and proteoglycans, to bear FcεRI on their surface, and to exhibit features of additional maturation when exposed to sodium butyrate *in vitro*.^{61,62}

I was delighted that Yukihiro Kitamura agreed to test whether the adoptive transfer of such *in vitro*-derived immature mast cells into WBB6F₁-W/Wⁿ mice might result in further maturation of these cells *in vivo* and subsequent selective repair of the mast cell deficiency of the recipient mice. This collaborative effort showed that the transfer of WBB6F₁-^{+/+} mouse bone marrow-derived cultured mast cells by i.v., i.p., or intradermal injections into WBB6F₁-W/Wⁿ mice results in the appearance of mast cells in their tissues, and such mast cells over time exhibit phenotypic features similar to those present in the corresponding anatomic sites in the wild-type mice.⁶³ Since that initial study, many groups have used such mast cell-engrafted mice (or, as we named them, mast cell knockin mice) (Figure 2) to analyze mast cell development, phenotype, heterogeneity, and function *in vivo*.^{14,71,72} This approach allows the transfer of wild-type mast cells, mast cells that were genetically manipulated, or mast cells derived from various mutant or transgenic mice into different genetically

Figure 2 Schematic for the generation of mast cell knock-in mice. **1:** MCs are generated from bone marrow cells (or other hematopoietic cells; eg, those in the fetal liver) from wild-type mice or mutant or transgenic mice with specific genetic alterations of interest.^{61–63} **2 and 3:** Alternatively, ESMCs can be generated from wild-type or genetically altered ES cells (2),^{64,65} or MCs can be transduced *in vitro* with shRNA to diminish expression of specific genes of interest (3).^{15,66} **4:** Such BMCs or ESMCs (or fetal liver-derived cultured MCs), or shRNA-transduced MCs can then be transplanted into MC-deficient *c-kit* mutant mice, such as WBB6F₁-Kit^W/Kit^{W-v} mice^{63,67} or C57BL/6-Kit^{W-sh}/Kit^{W-sh} mice,^{68,69} or into C57BL/6-Cpa3-Cre;Mcl-1^{fl/fl} mice⁷⁰ (informally called Hello Kitty mice), which have wild-type *c-kit*, to produce MC knock-in mice. Note: BMCs can be injected into genetically MC-deficient mice intravenously, intraperitoneally, or intradermally or into the joints or meninges, etc., but there is a more limited experience with the engraftment of other types of MCs, such as ESMCs than with BMCs. **5:** A suitable interval is then allowed for engraftment and phenotypic maturation of the adoptively transferred MCs (the length of this interval can be varied, based on the route of MC transfer, the anatomical site of interest, the particular biological response being analyzed, etc.). **6–8:** The importance of MC 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 MC-deficient mice (7), and selectively MC-engrafted mutant mice (MC knock-in mice) (8). The contributions of specific MC 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 MC knock-in mice engrafted with wild-type MCs versus MCs derived from mice or ES cells that lack or express genetically altered forms of such products or that were transduced with shRNA to silence the specific genes that encode these products. An important part of the analysis of the MC knock-in mice is to assess the numbers and anatomical distribution and, for certain experiments, aspects of the phenotype of the adoptively transferred MCs, because, depending on the type of *in vitro*-derived MCs used, the route of administration, and other factors, these may differ from those of the corresponding native populations of MCs in the corresponding wild-type mice.^{14,71,72} BMC, bone marrow derived cultured mast cell; ES, embryonic stem; ESMC, embryonic stem cell-derived cultured mast cell; MC, mast cell. Modified from Metz et al,⁷¹ with permission from John Wiley and Sons.

mast cell-deficient recipients. This experimental approach in turn allows *in vivo* comparison of the functions of normal mast cells to mast cells with altered function of various receptors, signaling molecules, or mediators. Moreover, Mindy Tsai⁶⁴ led a project that demonstrated that one also can generate mast cells suitable for *in vivo* transfer from embryonic stem cells, permitting the analysis *in vivo* of mast cells that lack products which, if absent in the germline, would result in embryonic or perinatal lethality.

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 used with C57BL/6-Kit^{W-sh}/Kit^{W-sh} mice, which have the advantage of being inbred, fertile, and not anemic.^{68,69,73} This approach also can be pursued with C57BL/6-Cpa3-Cre⁺-Mcl-1^{fl/fl} mice, which are mast cell deficient (and also have substantially diminished numbers and function of basophils) because of the lineage-restricted ablation of the antiapoptotic 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 (Figure 2).

New Models for Analyzing the Roles of Basophils and Mast Cells in Health and Disease

In addition to the models described in the section above, a large number of additional models are now available to study the biology of mast cells, basophils, or their mediators, including other strains of mice that exhibit constitutive or inducible deficiencies in populations of mast cells or basophils or mice that lack constitutive or inducible expression of various mast cell mediators or other molecules.⁷² As reviewed in detail elsewhere,^{72,74,75} the results from studies employing the currently available mouse models of mast cell and/or basophil deficiency should be carefully interpreted, because these models differ in their relative advantages and possible drawbacks. Moreover, the importance of the contributions of mast cells (or basophils) in individual biological responses may vary on the basis of the background strain of the mice and the features of the particular biological response being tested. For these reasons, investigators should consider the use of more than one type of genetic model to investigate the roles of mast cells (or basophils) and/or their individual products in biological responses *in vivo*.⁷²

Elucidating a Beneficial Role for Mast Cells in Enhancing Innate Resistance to Venoms

Early work by Higginbotham⁷⁶ and Higginbotham and Karnella,⁷⁷ performed long before mast cell-deficient mice had been reported, suggested that mast cells might be able to diminish the toxicity of certain venoms by degranulating and releasing heparin in response to venom exposure. However, my laboratory's work to investigate the possible roles of mast cells in innate resistance to animal venoms was sparked accidentally, by the results of a project initiated by Marcus Maurer.⁶⁵ We uncovered evidence that mast cell activation by ET-1 can initiate a homeostatic mechanism whereby proteases released by such activated mast cells degrade ET-1 and thereby diminish its potential toxicity *in vivo*.⁶⁵ With the use of mast cell knockin C57BL/6-*Kit*^{W-sh}/*Kit*^{W-sh} mice engrafted with ET_A receptor-deficient or wild-type mast cells derived *in vitro* from ET_A^{-/-} or ET_A^{+/-} embryonic stem cells, we found that mast cell activation of ET-1 via the ET_A receptor contributed to this effect.⁶⁵

We then wondered whether such mast cell-protease-dependent detoxification of a biologically active peptide was restricted to ET-1 or also occurred with other endogenous or exogenous peptides.¹⁵ By searching the literature, Martin Metz discovered that ET-1 was structurally similar to sarafotoxin 6b, one of the main toxins in the venom of the Israeli mole viper (*Atractaspis engaddensis*).^{78,79} Sarafotoxin 6b can induce activation of cells in the envenomated animals by binding to endothelin receptors.⁷⁹ Mast cells diminished the toxicity of sarafotoxin 6b, enhanced the survival of mice injected with that peptide, and, more importantly, also enhanced the survival of mice injected with the whole venom

of *A. engaddensis*.¹⁵ (Figure 3). Testing the ability of mast cells to influence the response to the whole venom was critical, because the snake does not envenomate its prey with a single toxin but with a complex mixture of toxins that can induce pathology by different mechanisms.⁸¹ Initial pharmacologic studies suggested that chymase was the critical mast cell protease in this setting,⁶⁵ but later work by our group¹⁵ and elegant studies by Hans-Reimer Rodewald and colleagues⁸⁰ indicated that carboxypeptidase A3 is the key mast cell-derived protease that detoxifies both ET-1 and sarafotoxin 6b. By performing experiments with mast cell knockin mice, we also found 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

In a project led by Mitsuteru Akahoshi and Chang Ho Song,¹⁸ we then analyzed whether mast cells might enhance innate resistance to another pair of biologically active peptides, the endogenous mammalian peptide vasoactive intestinal polypeptide, 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*). Testing of both mast cell knockin mice [including C57BL/6-*Kit*^{W-sh}/*Kit*^{W-sh} mice engrafted with wild-type versus chymase (mMCP4)-deficient mast cells] and mice that had mast cells but were genetically deficient in mMCP4⁸² or CPA3⁸⁰ or produced a catalytically inactive CPA3⁸⁰ showed that mast cells diminish the toxicity of vasoactive intestinal polypeptide, helodermin, and the whole venom of *H. suspectum*, an effect that largely or wholly depends on mast cell-derived MCP4 rather than CPA3.¹⁸ We also showed that mast cells, and MCP4, can contribute to enhanced innate resistance of mice to the venoms of an Old World and a New World scorpion, the deathstalker (*Leiurus quikestriatus hebraeus*) and the Arizona bark scorpion (*Centruroides exilicauda*), respectively.¹⁸

We think it unlikely that mast cells can enhance innate resistance to all animal venoms, and it is possible that future work will reveal that mast cell activation can increase rather than decrease the toxicity of some venoms. However, our evidence indicates that mast cells can increase the innate resistance of mice to a first exposure to the venoms of three species of poisonous snakes, the Gila monster, the honeybee, and two especially dangerous scorpions. Moreover, evidence from our studies of the Israeli mole viper and the Gila monster indicates that mast cells contain at least two different proteases, CPA3 and chymase (MCP4), which equip them to respond, after the mast cells are activated via cognate receptors that can bind either the endogenous or reptile-derived peptides, to high and potentially toxic levels of ET-1 and vasoactive intestinal polypeptide, respectively, and to high levels of the structurally similar peptides contained in the reptile venoms (sarafotoxin 6b and helodermin, respectively) (Figure 4). In either setting (ie, responses to potentially toxic endogenous peptides or to

Figure 3 Reduction of MC-associated CPA with the use of shRNA in MCs renders mice susceptible to S6b-induced and A.e.v.-induced morbidity and mortality. **A** and **B**: Changes in rectal temperature, levels of sarafotoxins (assessed with an ELISA for endothelin-1 that detects both intact S6b and a nicked S6b that retains biological activity⁸⁰) in the peritoneal lavage fluid (at time of death or at 60 minutes, whichever was sooner), and 24-hour survival in C57BL/6 wild-type mice or C57BL/6-*Kit^{W-sh}/Kit^{W-sh}* mice that were engrafted intraperitoneally with empty vector (empty vector MCs → *Kit^{W-sh}/Kit^{W-sh}*) or CPA shRNA (CPA shRNA MCs → *Kit^{W-sh}/Kit^{W-sh}*) expressing bone marrow–derived cultured MCs and then were injected 4 weeks later with 1 nmol S6b (**A**) or 10 µg A.e.v. (**B**). Data are expressed as means ± SE. ***P* < 0.01, ****P* < 0.005 versus C57BL/6 or empty vector MCs → *Kit^{W-sh}/Kit^{W-sh}* mice. A.e.v., *Atractaspis engaddensis* venom; CPA, carboxypeptidase A3; ELISA, enzyme-linked immunosorbent assay; MC, mast cell; S6b, sarafotoxin 6b; Modified from Metz et al¹⁵ with permission from the American Association for the Advancement of Science.

peptides in venoms), by undergoing degranulation and releasing proteases that can inactivate the critical peptides, the mast cell can help to restore homeostasis, albeit while also enhancing aspects of the local and perhaps systemic inflammatory response.

Notably, depending on the mammalian species, mast cells can contain several tryptases and chymases of distinct substrate specificity, as well as carboxypeptidase A3.^{13,72,83} Moreover, mast cells are positioned in large numbers in the skin, the most common site of envenomation. One of the reasons that the mast cells of various species contain several different proteases in their cytoplasmic granules may be the need to release a suite 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.

A Beneficial Role for IgE in Host Defense against Venom

Although innate defenses against envenomation are important, many animals and some humans may experience

multiple episodes of envenomation by arthropods such as bees, wasps, and scorpions or by reptiles. In mammals, venoms not only provoke an innate inflammatory response and pathology related to the biological activities of the venoms' toxins,^{85–87} but they also can induce allergic sensitization that is now known to be associated with the development of specific IgE antibodies.^{88–93} In some unfortunate individuals such IgE responses to venoms, by priming tissue mast cells and blood basophils to release mediators of allergy and anaphylaxis on subsequent venom exposure, put them at risk of developing potentially fatal episodes of anaphylaxis.^{3,7,23,93} But is it possible, in accord with Profet's toxin hypothesis of allergy,³⁹ that this same mechanism, involving IgE and mast cells, also can enhance host resistance to venoms?

Honeybee (*Apis mellifera*) venom consists of a mixture of cytolytic peptides (eg, melittin), enzymes (eg, phospholipase A₂; considered the main allergen in bee venom), hyaluronidase, neurotoxins, and bioactive amines⁸⁵ and accounts for most 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 Thomas Marichal and Philipp Starkl et al,⁹⁵ pursuing a project in my laboratory that had

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 & 80)	Helodermin (See Ref. 18)
(similar peptides) ↕ Potentially toxic endogenous peptide	ET-1 (endothelin-1) (See Refs. 15, 65 & 80)	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)

Figure 4 Mast cells can enhance innate resistance to high levels of endogenous peptides (helping to restore homeostasis) and to structurally similar peptides in reptile venoms. Mast cell cytoplasmic granules contain proteases (such as CPA and MCP4) that, on secretion by activated mast cells, can degrade certain endogenous peptides, such as ET-1 and VIP, respectively, and 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 that 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, which 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. The photograph of the Israeli mole viper is by Amikam Shoob, courtesy of Elazar Kochva, Tel Aviv University, and the photograph of the Gila monster is from reptiles4all and is used under license from Shutterstock Stock Photos (New York, NY).

been initiated by Martin Metz, found that IgE-associated Th2 immune responses against honeybee venom or Russell’s viper venom were able to increase host resistance to challenge with a potentially lethal dose of the venom.

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 type 2 immune responses against venoms [that include the development of antivenom 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

Our evidence showed that IgE antibodies were the critical elements of the acquired host resistance to honeybee venom. We found that most or all of the acquired resistance induced in mice by a single exposure to honeybee venom was transferable to naive mice with only 250 µL of serum from

honeybee venom-immunized mice (Figure 5, A and B). The ability of the immune serum to transfer enhanced resistance to naive mice was essentially lost when immune serum was depleted of IgE either by adding a neutralizing antibody to IgE^{35,100} or by heating (56°C, 1 hour, which eliminates the ability of IgE to bind to FcεRI and induces passive cutaneous anaphylaxis,¹⁰¹ whereas the function of other antibody classes, including IgG₁, remains unchanged¹⁰²) (Figure 5, C and D). In addition, the immune serum failed to transfer immunity to mice that lacked 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} (Figure 5, E and F).

We also found 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. The immune serum from wild-type mice could passively transfer enhanced resistance to honeybee venom to naive IgE-deficient mice, unless such immune serum was

Figure 5 Evidence that IgE antibodies and the Fc ϵ RI α and γ chains contribute to acquired enhanced resistance to the toxicity and lethality of BV. **A** and **B**: Passive immunization of naive C57BL/6 mice with serum of BV-immunized mice can increase their resistance to a subsequent challenge with a potentially lethal dose of BV. Donor C57BL/6 mice were injected with $2 \times 200 \mu\text{g}$ BV or PBS. Three weeks later, sera from PBS- and BV-injected mice were collected, and naive 10- to 11-week-old C57BL/6 mice were passively immunized by i.v. injection of each mouse with $250 \mu\text{L}$ pooled serum from PBS-injected (PBS-serum, white circles/dashed lines) or BV-immunized mice (BV-serum, red diamonds/dashed lines). Twenty-two hours later, all mice were challenged with $4 \times 200 \mu\text{g}$ BV. Age-matched C57BL/6 mice that were actively immunized 3 weeks earlier with $2 \times 200 \mu\text{g}$ BV (red diamonds/solid lines) or PBS (white circles/solid lines) were used as controls. Changes in body temperature (A) and survival (% of live animals) (B) were monitored at the indicated times. Data shown are pooled from two to four independent experiments.⁹⁵ **C** and **D**: The increased resistance of passively immunized C57BL/6 wild-type mice to challenge with a potentially lethal dose of BV depends on functional serum IgE. Donor C57BL/6 mice were injected with $2 \times 200 \mu\text{g}$ BV or PBS. Three weeks later, sera from PBS- or BV-injected mice (PBS-serum or BV-serum, respectively) were collected, and pooled BV-serum was processed *in vitro* to neutralize IgE function, either by heating or anti-mouse IgE treatment (heated BV-serum or anti-IgE BV-serum, respectively). Age-matched recipient mice were anesthetized and injected intravenously with serum 22 hours before challenge with $4 \times 200 \mu\text{g}$ BV. Changes in body temperature after challenge (C) and survival (D) were monitored at indicated time points. Data are pooled from independent experiments performed with different pools of serum.⁹⁵ **E** and **F**: Fc ϵ RI α and Fc ϵ RI γ are required for BV-immunized mice to exhibit increased resistance against a potentially lethal dose of BV. Donor C57BL/6 mice were injected with $2 \times 200 \mu\text{g}$ BV or PBS. Three weeks later, sera from PBS- or BV-injected mice were collected and pooled separately and transferred intravenously ($250 \mu\text{L}$ pooled serum per mouse) into recipient C57BL/6 wild-type mice, C57BL/6-Fc ϵ RI α ^{-/-} mice (that lack the signaling γ chain of Fc ϵ RI) or C57BL/6-Fc ϵ RI α ^{-/-} mice (that lack the IgE binding α chain of Fc ϵ RI) 22 hours before challenge with $4 \times 200 \mu\text{g}$ BV. Changes in body temperature (E) and survival (F) were monitored at the indicated times. Data are pooled from independent experiments performed with different pools of serum.⁹⁵ Data are expressed as means $\pm$ SEM. $n = 2$ to 4 independent experiments (A and B); $n = 14$ to 20 per group from 2 different pools of serum and a total of 4 independent experiments (C and D). Data are pooled from experiments obtained with two different pools of serum and a total of three to four independent experiments ($n = 8$ to 17 per group from 2 different pools of serum and a total of 3 to 4 independent experiments (E and F). P values are calculated by Student's t -test (A, C, and E) and Mantel-Cox test (B, D, and F). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ for the indicated comparisons. BV, honeybee venom; PBS, phosphate-buffered saline; Δ Temp, changes in body temperature. Adapted and modified from Marichal et al,⁹⁵ with permission from Cell Press.

first treated to neutralize IgE or impair its ability to bind to FcεRI. In addition, naive 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 wild-type mice, actually exhibited worse survival after challenge with a high dose of honeybee venom than mast cell-deficient mice that had received serum from phosphate-buffered saline mock-immunized C57BL/6 wild-type mice.⁹⁵ The latter finding suggested that mast cells can contribute to IgE-mediated acquired resistance to honeybee venom and can enhance the innate resistance of mice to a first exposure to that venom.¹⁵ Independent of our work, Palm et al¹⁰³ showed that mice immunized with the main allergen contained in honeybee venom, bee venom phospholipase A₂ (bvPLA₂), exhibited enhanced resistance to the ability of bvPLA₂ to induce hypothermia on its injection into mice and provided evidence that this enhanced immunity required B cells and was diminished significantly in mice that lacked the IgE-binding α chain of the FcεRI. Taken together, the two studies support the hypothesis that one physiologic function of IgE is to protect the host against noxious substances.

Conclusions

Cells with morphologic, biochemical, and functional properties of mammalian mast cells, including the production of histamine, heparin, and serine proteases, are present in the tissues of tunicates, whose ancestors appeared in evolution before the development of adaptive immunity.^{104,105} Such creatures also are reported to have cells resembling basophils.¹⁰⁶ 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 ability allowed them to become immunologically primed or sensitized to undergo activation for mediator release on encountering relatively small amounts of the antigen identified by their surface-bound IgE antibodies. The most extreme example of an IgE-associated immune response that results in the activation of mast cells (and basophils) is fatal anaphylaxis, in which the rapid systemic and extensive release of mediators stored in these FcεRI-bearing effector cells results in a catastrophic and quickly lethal outcome. As put more graphically by Hans-Reimer Rodewald and Thorsten Feyerabend,⁷⁵ in this setting the mast cell has become the immune system's loaded gun.

As noted in the above sections, studies in experimental animals (including those using mice genetically deficient in mast cells, basophils, or IgE) and observational and epidemiologic studies in humans strongly suggest that one beneficial role of IgE, mast cells, and basophils is to help to defend the host against ticks and other ectoparasites and to

diminish the numbers of parasites and burden of disease in subjects infected with certain helminths. However, in addition to parasites, vertebrates have been subjected to evolutionary pressure through millions of years of co-evolution with venomous arthropods and reptiles and other venomous species. We found that mast cells can enhance innate resistance of mice to four species of poisonous snakes, the Gila monster, two species of scorpions, and the honeybee and that mast cell proteases (specifically, carboxypeptidase A3 and the chymase MCP4) can contribute to such mast cell-dependent innate defenses by degrading toxins present in some of these venoms. We also found that the 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 naive mice.⁹⁵

Despite extensive research, several questions remain unanswered. i) 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 (eg, *ex vivo* studies indicate that mast cell-derived heparin, which is highly anionic, can bind and thereby reduce the toxicity of cationic toxins in Russell's viper venom⁷⁶)? ii) 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 production?¹⁰³ iii) 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 immunologic roles of mast cells, basophils, and IgE? and iv) Why do some subjects develop such severe IgE-dependent reactivity to venom that they are at risk of fatal anaphylaxis (an outcome far from a protective immune response)?

In considering the latter question, it should be noted that many individuals who develop type 2 immune responses to bee venom do not exhibit anaphylactic reactivity despite having venom-specific IgE antibodies.¹⁰⁷ In addition, 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.^{108–110} 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 elicited antigen is a toxin.

Only time will tell what the answers to these questions may be, but pursuing them will be fascinating, and may well reveal some surprises.

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