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Testing the "toxin hypothesis of allergy": Mast cells, IgE, and innate and acquired immune responses to venoms*

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Summary

Work in mice indicates that innate functions of mast cells, particularly degradation of venom toxins by mast cell-derived proteases, can enhance resistance to certain arthropod or reptile venoms. Recent reports indicate that acquired Th2 immune responses associated with the production of IgE antibodies, induced by Russell's viper venom or honeybee venom, or by a component of honeybee venom, bee venom phospholipase 2 ($bvPLA_2$), can increase the resistance of mice to challenge with potentially lethal doses of either of the venoms or $bvPLA_2$. These findings support the conclusion that, in contrast to the detrimental effects associated with allergic Th2 immune responses, mast cells and IgE-dependent immune responses to venoms can contribute to innate and adaptive resistance to venom-induced pathology and mortality.

Introduction

Venoms from diverse animal species, including honeybees, wasps, scorpions, ants, Portuguese man-of-war, snakes, lizards, and the platypus can directly induce mast cell (MC) activation and degranulation [1–4]. Venom-induced release of granule-associated mediators by MCs has been thought to contribute to the symptoms associated with envenomation because some of these MC-derived mediators can increase vascular permeability (enhancing systemic dissemination of venom toxins), promote local recruitment and activation of inflammatory cells, influence clotting and fibrinolysis, and induce shock [5]. Moreover, many components of venoms also are "allergens" that can induce host sensitization *via* induction of Th2 immune responses and production of venom-specific Immunoglobulin E

*An annotated bibliography with comments about some of the same articles which are cited and commented on in this review is scheduled to appear in the October, 2015 issue of *Experimental Dermatology*, to accompany a lecture that will be given by Stephen J. Galli at a meeting organized by the Fondation René Touraine, on "Mast cells and urticaria", that will be held in Paris, France, on December 4, 2015.

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(IgE). Indeed, humans that have been sensitized with venoms can develop MC- and IgE-associated allergic reactions, including fatal anaphylaxis, upon subsequent venom exposure [6–12].

Such findings have supported the conclusion that venomous animals exploit to their own advantage the biological activities of the host's MCs and IgE, recruiting these components of innate and adaptive immunity to increase the toxicity of the venom. In 1991, Margie Profet suggested an alternative interpretation of the evidence indicating that MCs and IgE participate in immune responses to venoms, proposing that these components of innate and adaptive immunity may function to enhance rather than impair host resistance to venoms and, potentially, other toxins [13]. We review herein recent lines of evidence from studies in mice supporting the conclusion that both the innate functions of MCs and IgE-dependent Th2 immunity can be beneficial rather than detrimental in host responses to the venoms of some arthropods or reptiles.

Mast cells in *innate* resistance to envenomation

Higginbotham suggested in 1965 and 1971 that MCs, which are numerous in the skin, might enhance resistance to environmental noxious insults, such as bee stings [2] or snake bites [14]. He reported evidence that heparin, a highly anionic proteoglycan stored in MC granules, can neutralize venom toxicity by binding highly cationic components of the venoms, such as melittin in bee venom. This work was done before knock-out or MC-deficient mice had been described, and even now it is difficult to analyze the role of MC heparin using genetic approaches because mice deficient in heparin express many other phenotypic abnormalities including reduced storage of proteases in MC granules [15].

However, the beneficial role of MCs in enhancing resistance to venoms hypothesized by Higginbotham was later supported by studies of innate responses to venoms or venom components in MC-deficient mice [1,4], mice lacking specific MC proteases [1,4], and mice in which the MC protease, carboxypeptidase A3 (CPA3), was rendered catalytically inactive [16]. For example, MC-deficient C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ mice (whose MC deficiency is caused by a mutation affecting expression of Kit, the receptor for the MC survival and maturation factor stem cell factor [4,17]) and C57BL/6-*Cpa3*-Cre^{+}-$Mcl\text{-}1^{fl/fl}$ mice [17] (whose marked MC deficiency and decreased numbers of basophils are not due to c-*kit* mutations [18]) were significantly *more* susceptible to challenge with a potentially lethal dose of honeybee (*Apis mellifera*) venom (BV) than either wild type or control mice (i.e., littermate $Kit^{+/+}$ and *Cpa3*-Cre^{+}-$Mcl\text{-}1^{+/+}$ mice) or MC-deficient mice whose skin had been engrafted with MCs derived *in vitro* from the corresponding wild type mice. These and other experiments provided compelling evidence that MCs can enhance *innate* resistance in mice to the morbidity and mortality induced by the whole venoms of the honeybee [4,17], three species of snakes (Israeli Mole Viper, western diamondback rattlesnake, and southern copperhead) [4], the Gila monster lizard [1], and two species of scorpions [1].

While it is possible that MC-derived heparin contributes to the ability of MCs to enhance innate resistance to some venoms (especially those which contain highly cationic toxins), it is now clear that at least two MC-associated proteases, mouse CPA3 and mouse MCPT4

(the mouse chymase with functional similarity to human MC chymase [15]) can have important roles in augmenting innate host resistance to certain arthropod or reptile venoms (Fig. 1). Pharmacological evidence and studies in mice containing MCs that had been treated with shRNA specific for CPA3 suggested that CPA3 is the critical protease responsible for reducing the toxicity of both the whole venom of the Israeli mole viper (*Atractaspis engaddensis*) and its major toxin, sarafotoxin 6b [4]. The essential role for CPA3 in degrading and enhancing resistance to sarafotoxin 6b was then demonstrated elegantly in experiments employing transgenic mice that produce only a catalytically inactive CPA3 [16]. Notably, both sarafotoxin 6b [4] and its structurally related mammalian molecule, the vasoconstrictor peptide endothelin-1 (ET-1), can induce MC degranulation in mice, and MCs [16,19] and MC-derived CPA3 [4,16] can enhance resistance to the morbidity and mortality associated with the toxicity of either ET-1 or sarafotoxin 6b.

The venom of the Gila monster (*Heloderma suspectum*) represents another example of a reptile venom that contains a MC-activating peptide, in this case, helodermin, which has structural and functional similarity to a mammalian peptide, i.e., vasoactive intestinal peptide (VIP). However, extensive evidence indicates that it is mouse MCPT4, not CPA3, which is important in diminishing the toxicity of *H. suspectum* venom, helodermin, and VIP, as well as certain scorpion venoms [1]. These findings support the hypothesis that MCs and MC-derived proteases can contribute to maintaining homeostasis in two distinct settings associated with high levels of peptides that can induce MC activation: 1) Degradation of potentially toxic *endogenous* peptides, such as ET-1 or VIP, by MCs activated by such peptides via innate receptors expressed on the MC surface; 2) Degradation of toxic *exogenous* peptides, such as sarafatoxin 6b or helodermin, by MCs activated when such peptides are recognized by the same innate receptors that bind the corresponding mammalian peptide (Fig. 1). In addition to MC-derived proteases and heparin, other MC-derived mediators also have the potential to enhance innate resistance to venoms. For example, mediators that increase local vascular permeability might help to elevate interstitial concentrations of circulating inhibitors of venom metalloproteases and other toxic venom components.

Mechanisms of mast cell activation in *innate* responses to envenomation

Multiple mechanisms can contribute to MC activation during envenomation. Some venoms contain peptides that are structurally similar to endogenous peptides (e.g., kallikrein, atrial natriuretic peptide, or vascular endothelial growth factor [20]) and that are recognized by innate receptors on the MC surface. For example, ET_A and ET_B recognize ET-1 and sarafatoxins [4,19] and $VPAC_1$ and $VPAC_2$ recognize VIP and helodermin [21]. Venoms also can contain cytolytic peptides, enzymes (particularly phospholipases, which are present in many venoms), bioactive amines, salts, neurotransmitters, etc. [22]. Cytolytic venom components can mediate cell lysis *directly* by integration into cell membranes and pore formation (e.g., melittin in BV and cytolytic peptides in scorpion venom) or *indirectly* (e.g., venom-derived PLA_2) by enzymatic cleavage of membrane phospholipids and generation of lysophospholipids [23].

Palm & Medzhitov showed that BV, primarily by its pore-forming peptide, melittin, can induce NLRP3 inflammasome dependent caspase-1 activation and IL-1/IL-1R dependent neutrophil recruitment at sites of BV injection [24]. Although neutrophil depletion did not diminish the pathology or hypothermia induced by western diamondback rattlesnake venom, neutrophils may contribute to tissue repair following envenomation. Experiments employing MC- & caspase-1 double deficient ($Kit^{W\text{-}sh/W\text{-}sh}$;$Casp1^{-/-}$) mice suggest that MCs may be involved in caspase-1 dependent protection from envenomation [24], but it is not yet clear whether the NLRP3 inflammasome is involved in venom-induced MC activation in tissues.

MCs can be activated to degranulate at sites of complement activation *via* C3a or C5a binding to C3aR or C5aR on the MC surface [25]. Since envenomation is often associated with complement activation [26], local production of C3a and C5a may contribute to MC activation at sites of envenomation.

IgE, FcεRI, and mast cells in *adaptive* immune resistance to envenomation

Allergies, characterized by acquired Th2 immune responses and the production of allergen-specific IgE antibodies [27–29], are widely considered misdirected and maladaptive immune responses, often against otherwise innocuous environmental agents [30,31]. The immediate hypersensitivity reactions which occur in sensitized individuals within moments of allergen exposure range from localized areas of swelling, erythema and itching (in response to cutaneous encounters with the allergen) to catastrophic and sometimes fatal anaphylactic reactions, that can be induced by animal venoms, as well as by foods, drugs, and other seemingly harmless compounds [32,33]. By contrast, IgE-associated Th2 responses are thought to contribute to host defense during infections with certain helminths and other parasites [29,34–36]. However, any other “physiological” roles of IgE have remained obscure.

In 1974, James H. Stebbings proposed that “a major function of immediate hypersensitivity reactions has been the protection of terrestrial vertebrates from the bites of, or invasion by, arthropods” [37]. He hypothesized three advantageous functions of immediate hypersensitivity: 1) the induction of avoidance behavior, 2) protection against immune injury caused by antigen-antibody complexes, and 3) inhibition of delayed hypersensitivity reactions, and also noted that some insects which may induce immediate hypersensitivity, such as tropical mosquitoes, are potential disease vectors [37]. Indeed, if IgE-dependent immediate hypersensitivity reactions to arthropod bites have effects that help hosts to experience fewer bites, this could contribute to diminished transmission of arthropod-borne diseases. In 1991, Margie Profet noted that the common feature of most allergens is their origin from sources (such as nuts, seafood, or venoms) which either might (e.g., foods) or always (e.g., venoms) contain toxins [13]. Profet proposed that allergic reactions (manifested as immediately occurring symptoms, such as coughing or diarrhea) evolved as defense mechanisms which allow the sensitized host to respond immediately to, and to eliminate, neutralize and/or avoid, noxious substances which might be indicative of potentially life-threatening situations [13]. However, Profet’s “toxin hypothesis” only recently gained wide attention [38]. Two recent studies of mice sensitized and challenged

with animal venoms [17] or a venom component [39] have now provided experimental support for key aspects of this hypothesis.

We showed that Th2 immunity induced in mice injected with sublethal amounts of the whole venoms of the honeybee or the Russell's viper (*Daboia russelii*) [17] significantly *enhanced* the survival of mice later challenged with potentially lethal doses of those venoms. In such Th2 responses to BV, experiments employing passive transfer of IgE-containing or IgE-depleted serum from BV-immunized to naïve mice, as well as studies in mice lacking IgE or the FcεRI α or γ chains, showed that both IgE and functional FcεRI are required for expression of enhanced acquired resistance to the morbidity and mortality induced by BV [17]. Palm *et al*. investigated Th2 responses to honeybee venom (BV) and several snake venoms, and showed that the Th2 immune responses induced by injections of BV phospholipase A_2 (bvPLA$_2$) *diminished* the drop in body temperature of mice challenged with a potentially lethal amount of bvPLA$_2$ [39]. This beneficial effect of bvPLA$_2$ immunization was significantly reduced in mice that lacked the FcεRI α chain, consistent with a role for IgEFc εRI interactions in enhancing resistance to bvPLA$_2$.

While the intrinsic toxicity of BV reflects the actions of multiple constituents, including cytolytic peptides (e.g., melittin), enzymes (including bvPLA$_2$ and hyaluronidase), neurotoxins and bioactive amines [40], by inducing the development of BV-specific IgE antibodies, honeybee stings can prime some unfortunate individuals to exhibit anaphylaxis in response to even a single sting [10]. In that setting, the pathology induced by the immune response far exceeds that induced by the intrinsic toxicity of the venom. BvPLA$_2$ is the most potent allergic protein in BV (although accounting for only about 12% of the weight of BV [40]) and its enzymatic activity (associated with the conversion of membrane phospholipids into arachidonic acid and lysophospholipids that cause cell lysis) is required for the induction of bvPLA$_2$-specific Th2 [39] and IgE responses [41].

MCs are major FcεRI-expressing IgE effector cells [28,42–44]. As discussed above, MCs contribute importantly to *innate* resistance to BV and other venoms and venom components [1,4,16]. We found in serum transfer experiments that MC-deficient mice that received immune serum collected from BV-immunized wild type mice exhibited significantly *worse* survival upon BV challenge compared to those which had been passively immunized with control serum from mock-immunized mice [17]. This result is consistent with the conclusion that MCs can contribute to IgE-mediated enhanced resistance to BV, and also suggests that, in the absence of MCs, components of BV-immune serum may actually exacerbate, rather than ameliorate, the adverse response to BV.

Conclusions

Work done independently in two groups now supports the conclusion that Th2 and IgE-associated immune responses can enhance resistance to whole BV [17] and bvPLA$_2$ [39]. We think that this likely reflects, at least in part, the ability of MCs bearing on their surface venom-specific IgE antibodies to respond more rapidly and perhaps more extensively to encounters with venom than do MCs which can respond to these venoms solely by innate mechanisms. Such venom-IgE-sensitized MCs also can be activated by lower concentrations

of venom than are needed to induce degranulation of naïve MCs [17]. Once MCs have been activated, whether by innate or IgE-dependent mechanisms (or by a combination of these mechanisms) and MC proteases and other granule contents have been exteriorized, then these mediators work according to chemical rules (e.g., based on the substrate specificity of the released enzymes) in an immunologically non-specific fashion to identify and degrade toxic components of the venom (Fig. 2).

In principle, IgE-enhanced recruitment of MC proteases, and other MC mediators which may have beneficial effects in countering the activities of multiple venom toxins, can occur even if the Th2 response induced by venoms results in the development of IgE antibodies specific for only some of the venom components: once MCs have been activated, the released proteases can degrade multiple venom components, not just the ones recognized by IgE. Moreover, such MC mediator-dependent neutralization of venom components may occur both locally at the site of envenomation and, in highly envenomated animals, perhaps systemically. Indeed, in the latter situation, the induction of IgE-dependent “anaphylaxis” (i.e., a strong systemic response induced by the activation of MCs in multiple anatomic compartments) may help to ensure that toxins distributed by the circulation beyond the site of envenomation also can be degraded by MC-derived proteases. From that perspective, perhaps even anaphylaxis can in some cases be “protective”, assuming of course that one survives it.

What next? We are only at the beginning of efforts to understand the beneficial roles of MCs and IgE in enhancing host resistance to venoms and other potentially toxic environmental challenges. There are many questions to answer. In how many ways can the immune response detect such challenges? Can other MC-derived mediators, beyond proteases and perhaps heparin, confer benefit in such settings? Can IgE enhance adaptive resistance to venoms by mechanisms other than those involving MCs? And what are the genetic or environmental factors which determine whether envenomated animals develop a propensity to exhibit anaphylaxis to tiny amounts of venom (amounts that would not intrinsically induce serious pathology) as opposed to a “protective” response that can help the host to resist the toxicity associated with large amounts of such venom [9–12]? It is well known that some individuals develop Th2 responses to specific venoms (such as BV) without exhibiting allergic reactions to that venom [45], and there is much evidence that Th2 responses are subject to intricate immune regulation that can down-regulate IgE-dependent reactivity to the inducing antigen [46], including BV [47–49]. Delineating the mechanisms underlying the development of potentially “anaphylactic” vs. “protective” Th2 immune responses, and the mechanisms which account for the innate and adaptive functions of MCs and IgE that enhance resistance to venom, may help in the development of better protocols for venom immunotherapy and in the prevention of catastrophic anaphylactic reactions to environmental substances, as well as in the design of improved therapeutics to treat envenomated people and other animals.

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immune hypersensitivity, rather than being purely deleterious for the host, evolved as defense mechanisms against a large array of environmental toxic substances, including venoms and plant compounds.

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triggering activation of caspase-1 and the subsequent secretion of IL-1β by macrophages. Activation of the inflammasome by bee venom induced caspase-1–dependent inflammation, including neutrophil recruitment to the site or envenomation, but the inflammasome was dispensable for the allergic response to bee venom. The authors found that caspase-1–deficient mice were more susceptible to the toxicity of bee and snake venoms, suggesting that a caspase-1–dependent innate immune response can enhance resistance to such envenomation.

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Highlights

- Mast cell activation can contribute to *innate* immunity to multiple animal venoms.
- Mast cell granule-associated proteases can degrade toxic components of venoms.
- Th2 immune responses can enhance survival in mice injected with certain venoms.
- IgE and FcεRI can be key elements of acquired Th2 immune resistance to venom.
- IgE-dependent mast cell activation can contribute to *acquired* resistance to venoms.

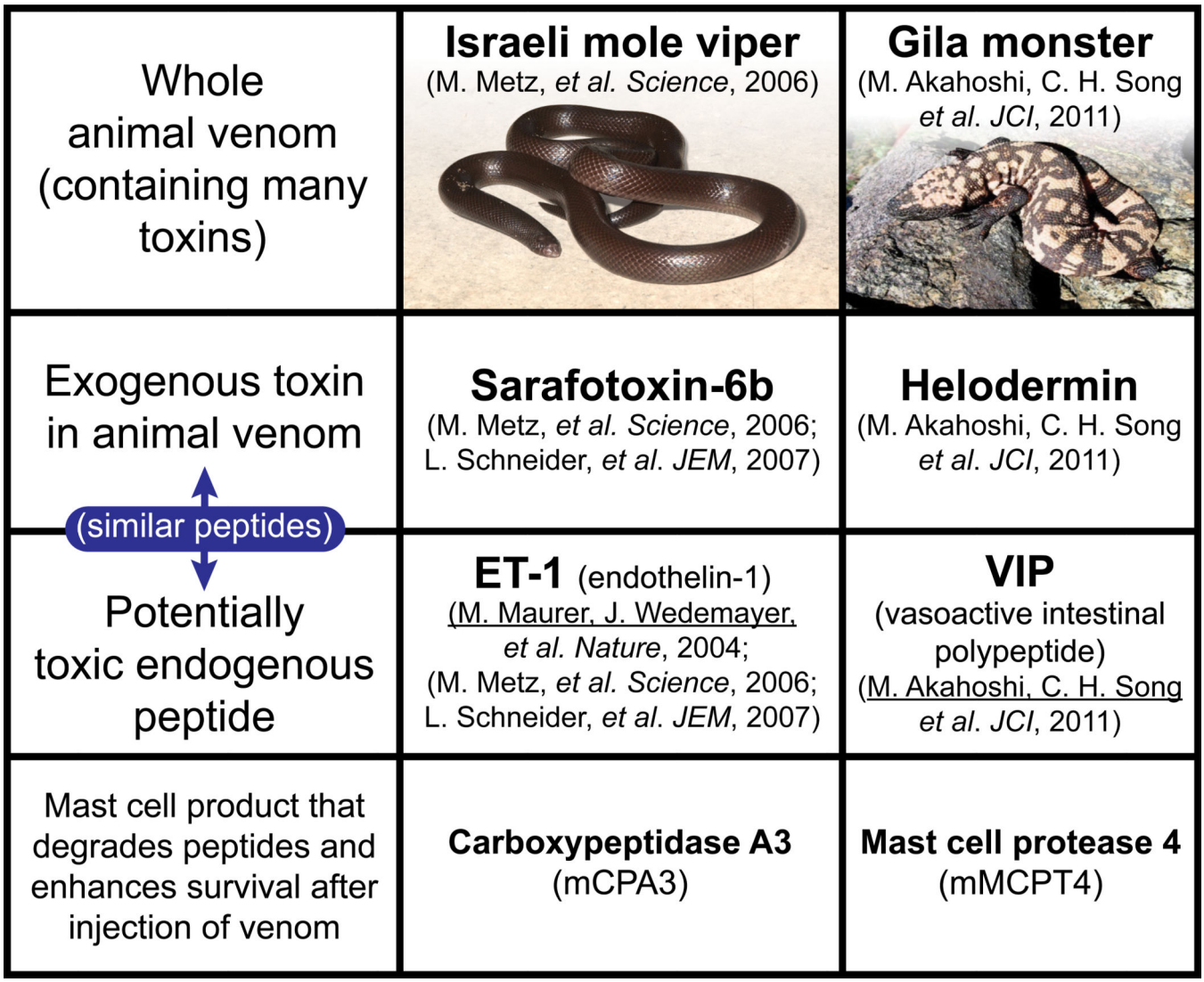

Figure 1. Mast cells can enhance resistance to both high levels of endogenous peptides (helping to restore homeostasis) and structurally similar peptides in reptile venoms

Mouse MC cytoplasmic granules contain proteases such as carboxypeptidase A3 (mCPA3) and mast cell protease 4 (mMCPT4) 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 thereby 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 increase levels of the

endogenous peptides. This is a modified version of Fig. 4 in the review: Rouse-Whipple Award Lecture: The mast cell-IgE paradox: From homeostasis to anaphylaxis, by Stephen J. Galli, reproduced with the permission of the publisher.

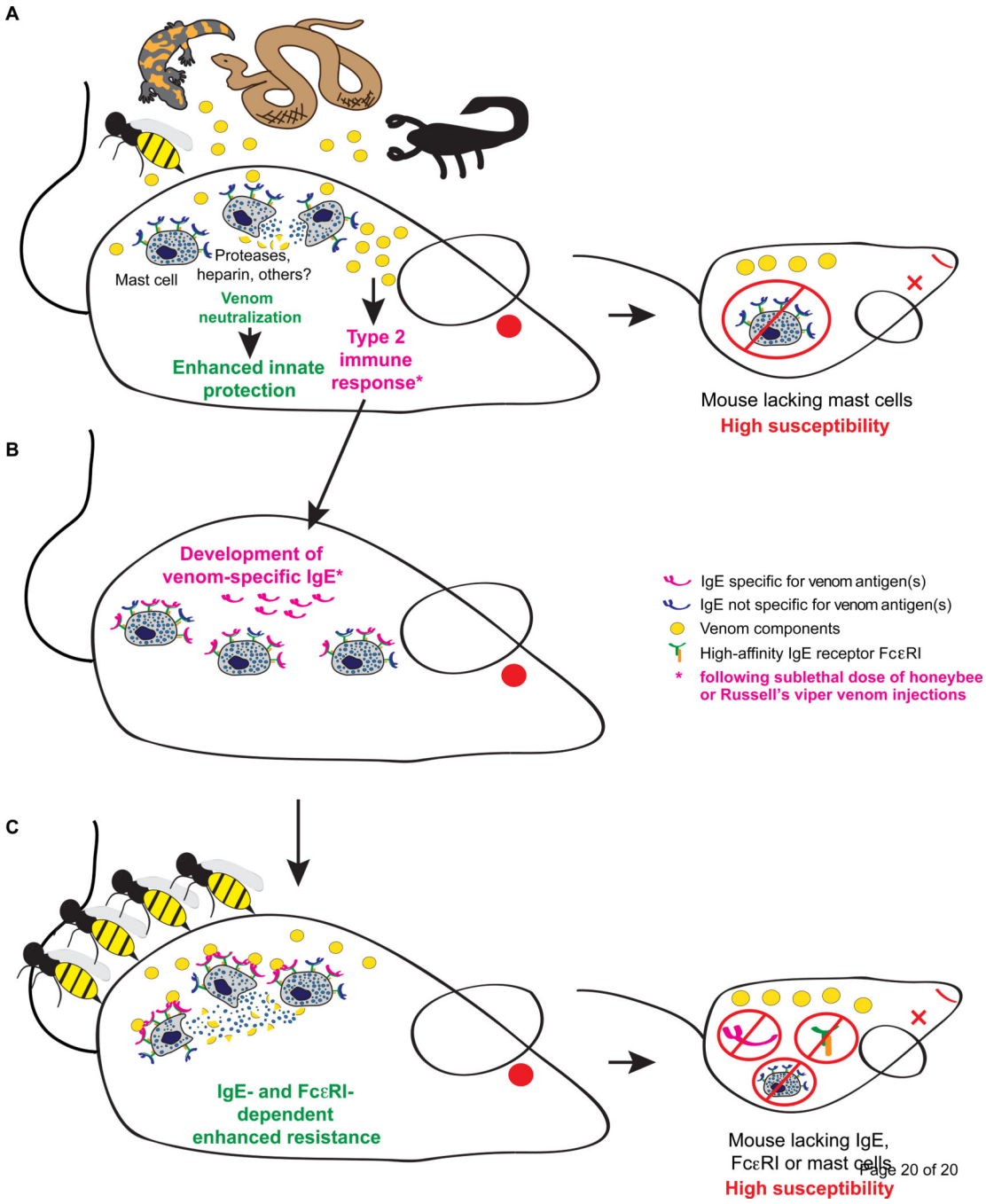

Figure 2. Innate and IgE/ FcεRI-dependent activation of mast cells by venom components can enhance resistance to a potentially lethal dose of whole venoms

(A) MCs can enhance *innate* resistance of mice to the morbidity and mortality induced by the whole venoms of the honeybee, three species of snakes (Israeli Mole Viper, Western diamondback rattlesnake, and Southern copperhead), the Gila monster lizard, and two species of scorpions through mechanisms that depend on the release of mediators that can neutralize toxic components of venoms.

(B) Injection of a sub-lethal dose of honeybee (BV) or Russell's viper venom (RVV) induces an adaptive type 2 immune response in mice that is associated with development of IgE antibodies that can increase the resistance of mice to a potentially lethal dose of the same venom. (C) The protective effect of the type 2 immune response against BV is mediated by IgE antibodies, FcεRI and mast cells.