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IgE Antibodies, FcεRIαand IgE-mediated Local Anaphylaxis Can Limit Snake Venom Toxicity

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Abstract

Background—Type 2 cytokine-related (i.e., type 2) immune responses associated with development of antigen-specific Immunoglobulin E antibodies (IgE) can contribute to pathology in allergic diseases and to fatal anaphylaxis. However, recent findings in mice indicate that IgE also can enhance defense against honeybee venom.

Objective—We tested whether IgE antibodies, IgE-dependent effector mechanisms, and a local anaphylactic reaction to an unrelated antigen can enhance defense against Russell's viper venom (RVV) and determined whether such responses can be influenced by immunization protocol or mouse strain.

Methods—We compared the resistance of RVV-immunized wild-type, IgE-deficient, and *Fcer1a*-deficient mice following injection of a potentially lethal dose of RVV.

Results—A single prior exposure to RVV enhanced the ability of wild-type mice, but not mice lacking IgE or functional FcεRI, to survive challenge with a potentially lethal amount of RVV. Moreover, IgE-dependent local passive cutaneous anaphylaxis in response to challenge with an antigen not naturally present in RVV significantly enhanced resistance to the venom. Finally, we observed different effects on resistance to RVV or honeybee venoms in BALB/c *versus* C57BL/6 mice which had received a second exposure to that venom prior to challenge with a high dose of that venom.

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Conclusion—These observations illustrate the potential benefit of IgE-dependent effector mechanisms in acquired host defense against venoms. The extent to which type 2 immune responses against venoms can decrease pathology associated with envenomation seems to be influenced by the type of venom, the frequency of venom exposure, and the genetic background of the host.

Keywords

Acquired resistance; allergy; *Daboia russelii*; Russell's viper; FcεRIα; honeybee; immunoglobulin E; toxin hypothesis; venom; mast cells; type 2 immunity

Introduction

Venoms are complex mixtures of toxic molecules[1-3] employed by many different animal species to fulfill functions of deterrence, defense, and/or predation[4, 5]. Millions of years of co-evolution with venomous animals have allowed certain mammals, including those that eat or are the prey of venomous creatures, to develop innate defense mechanisms that can increase their basal (or “innate”) resistance against venoms and their toxins. Such specialized defense strategies include producing circulating serum proteins that efficiently neutralize venom components[6, 7] and conserving mutations, e.g., in proteins targeted by toxins[8], which confer increased resistance to that toxin.

We and others have been interested in the possibility that mast cells (MCs) can represent an important component of the innate defense of vertebrates to animal venoms. MCs populate virtually all vascularized mammalian tissues[9-11]. When appropriately activated, MCs can release cytoplasmic granules containing a broad spectrum of pre-formed mediators into the surrounding tissues[11]. Notably, many components of animal venoms can induce such MC degranulation[12] and some mediators stored in MC granules have the ability to neutralize the toxicity of components of animal venoms[13-17]. Higginbotham and colleagues showed that the venoms of the honeybee[15] and the highly poisonous[18, 19] Russell's viper[14] can induce degranulation of mouse MCs *in vivo*[14, 15], and that the toxicity of those venoms was significantly reduced upon their *ex vivo* incubation with heparin, the serglycin proteoglycan stored in MC cytoplasmic granules[20]. More recently, mice deficient in MCs or certain MC-associated proteases were used to show that MCs can importantly contribute to innate host defense against venoms[13, 16, 17, 21], or toxic venom components[13, 16, 17], of honeybees[16, 21], two scorpions[13], and various reptiles[13, 16, 17].

Since IgE antibodies can enhance MC sensitivity and responsiveness against specific antigens, and in light of evidence that MCs can enhance innate resistance to venoms[13, 16, 17, 21], Profet[22], Metz et al.[16], and Palm et al.[23] speculated that IgE antibodies may also play a protective role in acquired resistance to venoms. However, it is well known that humans and other mammals which develop IgE antibodies to venom components from honeybees[2, 24], reptiles[25-29], or other animals[30-33] can exhibit anaphylaxis, a catastrophic and potentially fatal acute allergic reaction, upon subsequent venom exposure[34, 35]. Such observations suggested that the development of an acquired T helper cell type 2 (T_H2 or

type 2) immune response and associated IgE directed against venom components probably would increase, not decrease, the pathology associated with envenomation.

Recently, our group[21] and Palm et al.[36] reported that the development of a type 2 immune response to honeybee venom (BV)[21] or BV phospholipase A_2 (bvPLA$_2$)[36] could increase the resistance of mice (as quantified by body temperature[21, 36] and/or survival[21]) against a near-lethal dose challenge of whole BV[21] or bvPLA$_2$[36]. This effect was dependent on the high affinity IgE receptor (i.e., FcεRIα[21, 36]) and IgE antibodies[21]. In addition, we also observed that injection of mice with sublethal amounts of Russell's viper venom (RVV), a snake venom of high clinical relevance[18, 19], induced a type 2 immune response that enhanced the survival of mice injected with a potentially lethal amount of that venom[21].

In the present study, we aimed to define the importance of IgE antibodies, FcεRIα, FcεRIα$^+$ IgE effector cells, and local IgE-mediated MC activation in the orchestration of systemic resistance against RVV. In addition, we evaluated the influence of repeated exposure to venom and the genetic background of the host on acquired protection against challenge with a potentially lethal amount of RVV or BV.

Methods

Mice

All animal care and experiments were carried out in accord with current National Institutes of Health guidelines and with the approval of the Stanford University Institutional Animal Care and Use Committee. Age-matched 5 to 7 week-old WT C57BL/6J or BALB/cJ female mice were purchased from Jackson Laboratories. All transgenic mouse strains were bred and housed with the respective (in house-bred) control mice in the Stanford Animal facilities under specific pathogen free conditions. Details regarding transgenic strains can be found in this article's Online Repository at www.jacionline.org.

Reagents

Russell's viper (*Daboia russelii*) venom was obtained from Sigma (Lots SLBB5602V and SLBK7058V). Details regarding additional reagents can be found in this article's Online Repository at www.jacionline.org.

Venom injections

Briefly, mice were shaved at the injection sites 24 h before injections and were consistently treated in the morning (without anesthesia) by administering subcutaneous (s.c.) injections of 50 μL PBS alone or containing indicated amounts of RVV or BV. Additional details regarding injections, mouse handling, quantification of scratching behavior and descriptions of experiments involving serum transfer or multiple exposure to venoms prior to high dose venom challenge are provided in this article's Online Repository at www.jacionline.org.

Other methods

Detailed descriptions of the following methods are provided in the Online Repository at www.jacionline.org: histology and assessment of MC degranulation; analysis of skin and

white blood cells by flow cytometry; measurement of RVV-specific IgG_1 and IgE, BV-specific IgG_1, $bvPLA_2$-specific IgE and total IgE antibodies; anti-dinitrophenol-conjugated human serum albumin (DNP-HSA)-specific IgE-dependent passive cutaneous anaphylaxis; antibody-mediated neutrophil depletion; generation and degranulation analysis of bone marrow-derived cultured mast cells.

Statistical analysis

Statistical tests were performed using GraphPad PRISM 6 software. Two-tailed Student's *t*-test (unpaired), Mann-Whitney test, Mantel-Cox, or Chi-Square tests were performed as noted in the figure legends. ns, not significant ($P>0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Results

Mast cells rapidly degranulate upon injection of RVV and contribute to enhanced innate resistance to RVV

Injection of RVV s.c. into naïve C57BL/6 WT mice elicited intense scratching of that site (data not shown), rapid degranulation of skin MCs (Fig 1, A), local hemorrhage (Fig 1, B), and tissue infiltration with neutrophils and basophils (Fig 1, C,D), whereas the small numbers of eosinophils at such sites were not significantly different in sites injected with RVV *versus* PBS (data not shown). Systemically, RVV injection induced an increased percentage of blood neutrophils (see Fig. E1 in the Online Repository) and marked hypothermia (Fig 1, E). However, almost all mice appeared to recover fully within 24 h (Fig 1, E-F). Pre-treatment of C57BL/6 and BALB/c mice with the H_1 anti-histamine, triprolidine, but not with the platelet-activating factor (PAF) receptor antagonist, CV-6209, significantly decreased RVV-induced hypothermia without affecting mortality (Fig. 1, G-H and see Fig. E2 in the Online Repository). However, in C57BL/6 mice, combined treatment with the anti-histamine and PAF receptor antagonist did not protect against RVV-induced hypothermia and significantly increased RVV-induced mortality (Fig. 1, G-H), while such treatment decreased hypothermia but did not influence mortality in RVV-injected BALB/c mice (see Fig. E2 in the Online Repository). These findings suggest that there might be strain-dependent differences in the mechanisms contributing to responses to RVV in naïve mice.

We next evaluated the possible contributions of MCs, basophils and neutrophils to the type 2 humoral response induced by RVV. Injection of a sub-lethal dose of RVV in basophil deficient *Mcpt8-Cre^{het};DTA$^{fl/-}$* mice or MC- and basophil-deficient *Cpa3-Cre^{+};Mcl-1$^{fl/fl}$* mice (which are markedly deficient in MCs and have an ~75% reduction in blood basophils[37]) induced serum levels of IgG_1 and IgE antibodies not significantly different from those in the corresponding littermate controls (see Fig. E3, B-E in the Online Repository). Interestingly, anti-GR-1-treated neutrophil-depleted C57BL/6 mice developed similar levels of IgG_1 antibodies but significantly higher levels of IgE antibodies than did the isotype control antibody-treated mice (see Fig. E3, G-H in the Online Repository). These results provide evidence that neither mast cells, basophils nor neutrophils are necessary for the induction of a type 2 humoral immune response to RVV.

We next evaluated the possible contribution of MCs to innate resistance against RVV by testing two different types of MC-deficient mice (Fig 2, A). C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice virtually lack MCs (but exhibit moderately increased numbers of blood basophils[38]) due to a mutation in c-*kit*, the gene encoding stem cell factor receptor[39, 40]. The MC deficiency of C57BL/6-*Cpa3- Cre*$^{+}$*;Mcl-1*$^{fl/fl}$ mice is independent of c-*kit* and accompanied by decreased blood basophil numbers[37]. We found that each type of MC-deficient mouse exhibited significantly more susceptibility to RVV toxicity, assessed by extent of hypothermia (Fig 2, B-E) and/or survival (Fig 2, C-F), than did the corresponding control mice. MC-deficient mice also exhibited an almost complete absence of the scratching that was elicited in control mice (Fig 2, D-G).

IgE- and FcεRIα-dependent effector mechanisms contribute to increased survival of mice challenged with RVV

To assess whether IgE antibodies can contribute to acquired host resistance to RVV[21], we injected a low dose of RVV into IgE-deficient C57BL/6-*Igh7*$^{-/-}$ and IgE-sufficient C57BL/6-*Igh7*$^{+/+}$ mice (Fig 3, A). RVV induced RVV-specific IgG_1 antibodies in both *Igh7*$^{-/-}$ and *Igh7*$^{+/+}$ mice (Fig 3, B), but no detectable serum IgE in *Igh7*$^{-/-}$ mice (Fig 3, C). When challenged s.c. with a potentially lethal dose of RVV 3 weeks after their first exposure to RVV, C57BL/6-*Igh7*$^{+/+}$ mice, but not C57BL/6-*Igh7*$^{-/-}$ mice, exhibited enhanced resistance to the RVV-induced hypothermia and mortality (Fig 3, D-E). The same was true for the comparison between IgE-deficient and IgE-sufficient BALB/c mice (see Fig E4 in the Online Repository).

Serum transfer studies also supported a critical role for IgE antibodies in acquired resistance to RVV; enhanced protection could be transferred passively to naive C57BL/6 mice (Fig 3, F) by injecting them with 250 μl of serum collected from RVV-exposed WT donor mice (RVV-serum) that contained significantly increased levels of RVV-specific IgG_1 and IgE antibodies (Fig 3, G-H, respectively), but not with the same amount of serum obtained from PBS mock-immunized mice (PBS-serum) (Fig 3, F, I-J). Moreover, RVV-serum from WT mice lost its protective potential when the contained IgE antibodies were neutralized either by heating (which destroys the ability of IgE to bind to FcεRI and induce passive cutaneous anaphylaxis without affecting the function of other antibody isotypes[41, 42]) or treatment with an anti-IgE antibody (Fig 3, F, I -J).

Immune functions of IgE are primarily mediated by effector cells, including MCs and basophils, which express FcεRI[43, 44]. Both C57BL/6-*Fcer1a*$^{-/-}$ mice (that lack the IgE-binding component of FcεRI [i.e., FcεRIα]) and WT animals developed similar type 2 humoral responses after s.c. injection of RVV (Fig 4, A-C), but enhanced resistance to RVV challenge could only be detected in mice expressing the complete IgE receptor (Fig 4, D-E). Furthermore, in passive immunization experiments, C57BL/6-*Cpa3-Cre*$^{+}$*;Mcl-1*$^{fl/fl}$ mice exhibited no difference in survival after RVV challenge whether they had received untreated RVV-serum from C57BL/6 WT mice (which contained functionally active venom-specific IgE antibodies) *versus* control serum from PBS-mock-sensitized C57BL/6 WT mice (Fig 4, F-H).

Taken together, these results demonstrate that IgE antibodies and FcεRIα-bearing effector cells contribute importantly to the acquired resistance of RVV-immunized mice against a high dose RVV challenge.

A local anaphylactic reaction to an unrelated antigen can increase survival of mice challenged with a potentially lethal amount of RVV

Immunization with honeybee venom-derived PLA_2 (i.e., $bvPLA_2$), which represents approximately 10% of the dry weight of whole BV[45], can reduce the toxicity-related hypothermia induced by subsequent challenge of the mice with a high dose of the same allergen in an antibody- and FcεRIα-dependent manner[36]. However, it is 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 used a well-characterized monoclonal mouse anti-dinitrophenol (DNP) IgE antibody[46], which can sensitize mouse MCs to degranulate in response to challenge *in vivo* with DNP coupled to human serum albumin (DNP-HSA)[47, 48]. Specifically, we passively sensitized WT C57BL/6 and BALB/c mice against DNP-HSA by s.c. injections of anti-DNP IgE (or with anti-DNP IgG_1 or IgG_{2b} as controls), or mock-sensitized them with saline, then challenged the mice s.c. at the same site 24 h later by injecting a mixture of RVV and DNP-HSA (Fig 5, A). 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). We found that pre-sensitization with anti-DNP IgE significantly increased the resistance of C57BL/6 (Fig 5, B,C) or BALB/c (see Fig E5, H-I in the online repository) mice to challenge with a potentially lethal amount of RVV admixed with DNP-HSA. However, pre-sensitization of mice with anti-DNP IgG_1 or IgG_{2b}, DNP-specific IgG isotypes with the capacity to activate effector cells via Fcγreceptors[49], 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 5, B,C). Compared to passive sensitization with a 10 fold higher amount of anti-OVA IgE, anti-DNP IgE significantly enhanced the survival of IgE-deficient mice challenged with a potentially lethal amount of RVV admixed with DNP-HSA (Fig E6). By contrast, IgE-deficient mice passively sensitized with a 10:1 mixture of anti-OVA IgE and anti-DNP IgE exhibited a level of survival that was intermediate between that observed in mice which received either anti-DNP IgE alone or anti-OVA IgE alone (Fig E6, C). This result suggests that the effect on survival of antigen-specific IgE in this model may depend on the proportion of antigen-specific vs. antigen non-specific IgE on FcεRI-bearing effector cells.

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, 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.

Influence of venom type, genetic background, and venom exposure protocol on the protective effects of type 2 immune responses

IgE-associated type 2 immune responses induced by a single exposure to honeybee venom[21] or Russell's viper venom (this study) can increase the resistance of C57BL/6 or BALB/c mice to challenge with a potentially lethal amount of that venom. However, in nature, some animals may be exposed to the same venom more than twice. To analyze the potential effects of multiple venom exposures on acquired resistance to that venom, we injected mice s.c. with RVV (or PBS as a control) once at day 0, then some RVV-injected mice received a second RVV s.c. injection on day 21 (or got PBS as a control), and then all mice were challenged with a high dose of RVV at day 42 (Fig 6, A). C57BL/6 or BALB/c mice that had received 2 prior RVV injections (RVV-RVV mice) had significantly higher levels of RVV-specific IgG_1 (Fig 6, B-C), total IgE (Fig 6, D-E), and RVV-specific IgE (Fig 6, F-G) at day 35 than did the mice that received only a single RVV injection (RVV-PBS mice). Both RVV-RVV and RVV-PBS C57BL/6 mice developed less hypothermia upon RVV challenge than did control mice that had received two mock immunizations with PBS (PBS-PBS mice) (Fig 6, H). However, while survival of C57BL/6 mice injected once or twice with RVV was similar, the survival of the RVV-RVV mice did not quite achieve statistical significance *versus* that in the pooled PBS-PBS group ($P = 0.07$) (Fig 6, J). In BALB/c mice, animals injected once or twice with RVV were significantly more resistant than the PBS-PBS control mice to the hypothermia and the mortality induced by challenge with a potentially lethal dose of RVV (Fig 6, I-K).

We also tested the consequences of a second exposure to BV on responses to challenge with a potentially lethal amount of BV in C57BL/6 *versus* BALB/c mice (Fig 7, A). Serum levels of BV-specific IgG_1 (Fig 7, B,C), total IgE (Fig. 7, D,E) and $bvPLA_2$-specific IgE (Fig 7, F,G) were significantly higher in the serum of C57BL/6 mice that had received 2 exposures to BV (BV-BV mice) as compared to mice that had received only one (BV-PBS mice), whereas the differences in antibody levels in the two corresponding groups of BALB/c mice only were statistically significant in the case of $bvPLA_2$-specific IgE. C57BL/6 mice that had been injected once with BV prior to potentially lethal challenge showed significantly increased survival as compared to PBS-PBS control animals, confirming our prior findings[21], but this was not true for the C57BL/6 mice which were injected twice with BV prior to high dose BV challenge (Fig 7, J). Moreover, these BV-BV C57BL/6 mice exhibited a drop in body temperature in response to high dose BV challenge that was significantly more profound than that observed in the BV-PBS mice over the entire first 3 h of the response and that was even significantly worse than that of the PBS-PBS mice at 15 min after BV challenge (Fig 7, H). In contrast to the results with C57BL/6 mice, in BALB/c mice challenged with high dose BV, hypothermia was not significantly exacerbated in BV-BV *versus* BV-PBS mice (Fig 7, I) and survival was significantly enhanced by two BV exposures whereas the effect on survival did not reach statistical significance in the BVPBS mice ($P = 0.1$) (Fig 7, K). Notably, the strain-dependent differences observed in the responses to high dose BV in mice immunized once or twice with low dose BV did not appear to reflect differences in the ability of IgE antibodies from these mice to sensitize MCs to degranulate in response to BV challenge *in vitro* (see Fig E7 in the online repository).

Discussion

Antigen-specific IgE antibodies and FcεRI-expressing effector cells constitute a sensitive, specific, and powerful module of acquired immunity that can respond within minutes to exposure to small amounts of antigen by initiating local or systemic inflammatory reactions[9, 11]. It appears plausible that this rapid and efficient, but also potentially dangerous, effector mechanism evolved primarily to operate in situations that represent a substantial threat for the organism. In her “toxin hypothesis of allergy”, Margie Profet proposed that toxins and venoms represent examples of such substantial threats and that “allergic reactions” originally evolved as immune defense mechanisms against such noxious substances[22].

Recently, our lab[21] and others[36] provided *in vivo* experimental evidence that IgE antibodies can indeed contribute to protective immunity in mice against either whole BV[21] or the potentially toxic BV enzyme, bvPLA$_2$[36]. The results of the current study indicate that acquired IgE-mediated immune resistance is not restricted to BV, but can also be deployed as a potent adaptive immune defense mechanism against a reptile venom of high clinical relevance[19]. Notably, the immunization and challenge doses of RVV used in this study (25 μg and 50-100 μg, respectively), in relation to the body weight of a mouse, are similar to the amounts of venom that a human might be exposed to if bitten by a Russell's viper[19]. Taken together, our findings support the idea that IgE antibodies and FcεRIα-bearing effector cells may constitute part of a general defense strategy against animal venoms, in addition to having roles in host responses to certain macroparasites[50].

We also found that a local IgE-dependent reaction to an unrelated antigen (i.e., DNPHSA) not ordinarily contained in RVV can enhance the survival of mice subjected to challenge with a potentially lethal amount of RVV. Our results thus support the conclusion that mounting an IgE-dependent reaction to a single antigen can be sufficient to enhance host resistance to the complex mixture of toxins contained in the venom[51]. The effector mechanisms involved in such enhanced resistance remain to be defined, but may include MC-mediated venom detoxification[13-17] and the local dilution and/or interference with the systemic spread of the toxins[22, 23].

Ever since it was discovered that IgE antibodies can mediate anaphylactic reactions[52-55], the development of IgE antibodies specific for certain antigens, including components of venoms[25, 26, 29, 31, 33, 56, 57], has primarily been regarded as a risk factor for the development of deleterious IgE-mediated hyperreactivity upon subsequent antigen exposure. Yet a recent survey of more than 7,000 German adults[58, 59] showed a prevalence of 22.6 % for sensitization (i.e., having specific serum IgE antibodies) against hymenoptera (wasp and bee) venom in the general public[58], while the lifetime prevalence of diagnosed insect venom allergy in that group is only 2.8 %[59]. Indeed, it is well known from other studies that the vast majority (~80%) of people who have demonstrable IgE antibodies specific for hymenoptera venoms have no history of manifesting systemic reactions to such venoms[56, 60] and that the presence of antigen-specific IgE antibodies, taken in isolation, is not predictive of severe clinical reactivity to the recognized antigens[61-66]. It is therefore possible that, in

some humans, the presence of anti-venom IgE antibodies may be beneficial, e.g., by decreasing venom toxicity and tissue damage upon subsequent venom exposure.

It is thought that multiple factors, such as differences in pathogen exposure during childhood, the characteristics of the host's microbiome, and many other environmental influences, as well as genetic background and the nature and frequency of exposure to potential allergens, can contribute to the variation in individual susceptibilities to develop clinical allergies[67-70]. Here we compared the resistance of C57BL/6 and BALB/c mice to RVV or BV following one *versus* two sublethal exposures to the same venom. In contrast to BALB/c mice, C57BL/6 mice that were immunized twice with BV rapidly developed increased hypothermia upon subsequent BV challenge. Importantly, such twice-immunized C57BL/6 mice, in striking contrast to singly immunized C57BL6 mice or twice-immunized BALB/c mice, did not exhibit enhanced resistance against high dose BV challenge. Taken together, our data indicate that, depending on the mouse strain and the type of venom, a second exposure to venom can either increase (BV in BALB/c mice) or eliminate (BV in C57BL/6 mice) the enhanced protection to venom challenge that is observed after a single exposure to that venom. While many factors might contribute to such strain-dependent differences, including genetically-determined differences in end organ sensitivity to MC-derived mediators[71-74], our data suggest that such factors probably don't include differences in the ability of IgE antibodies from these mice to sensitize MCs to degranulate in response to BV challenge.

Our findings are consistent with the hypothesis that the co-evolution of mammals with venomous animals provided positive evolutionary pressure to conserve IgE antibodies and IgE-effector cells as survival advantages. However, it seems likely that sustaining the beneficial functions of this “allergy module” of immunity critically requires regulatory mechanisms which can keep this potentially dangerous effector mechanism under tight control. We therefore speculate that anaphylaxis represents only the most extreme end of a spectrum of acquired T_H2 immunity to venom and that appropriately regulated T_H2 immune responses can actually enhance resistance, rather than susceptibility, to venoms.

In fact, the occurrence of potentially dangerous allergic T_H2 responses in some individuals may represent the price paid to maintain, for the species, the benefits of IgE-associated T_H2 immune responses. For example, beekeepers, who are frequently exposed to bee venom, can exhibit high levels of BV-specific IgG and IgE antibodies, associated, in some of these individuals, with the danger of anaphylaxis[75]. However, in many beekeepers, exposure to multiple bee stings as the season progresses induces the development of BV-specific, IL-10-producing, inducible type 1 T regulatory (T_R1) cells, which suppress T cell responses to BV *in vitro* and which, *in vivo*, may contribute to the observed reduction in cutaneous late phase responses to bee stings which occur as the beekeeping season progresses[76]. Mechanisms of antigen-induced, regulatory T cell-dependent immune tolerance also are thought to contribute to the success of venom specific immunotherapy in patients with hymenoptera venom allergy[77]. It is therefore tempting to speculate that IgE-dependent enhanced resistance to the toxicity of BV may represent an initial phase of a beneficial adaptive immune response to BV which, in individuals frequently exposed to the venom, then can be supplemented or supplanted by T regulatory cell-dependent immune tolerance to BV, one

important function of which is to restrain the development of an overly excessive, and therefore potentially dangerous, IgE response to BV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMCMCs	bone marrow-derived cultured mast cells
BV	honeybee venom
bvPLA$_2$	honeybee venom phospholipase A$_2$
DNP	dinitrophenol
DNP-HSA	dinitrophenol-conjugated human serum albumin
IgE	Immunoglobulin E (antibody)
MC(s)	mast cell(s)
PAF	platelet activating factor
RVV	Russell's viper venom
s.c.	subcutaneous
T$_H$2	T helper cell type 2
WT	wild type

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Key Messages

- IgE and IgE effector mechanisms can limit Russell's viper venom toxicity in mice.
- A local anaphylactic reaction elicited by an unrelated antigen at the site of Russell's viper venom injection can increase resistance against that venom.
- The extent of IgE-associated acquired resistance to venom can be influenced by venom type, mouse genetics, and the number of exposures to that venom.

Capsule Summary

IgE and IgE effector cells, and local anaphylactic reactions, can increase resistance to a snake venom in mice. Such acquired venom resistance is influenced by type of venom, host genetics, and number of venom exposures.

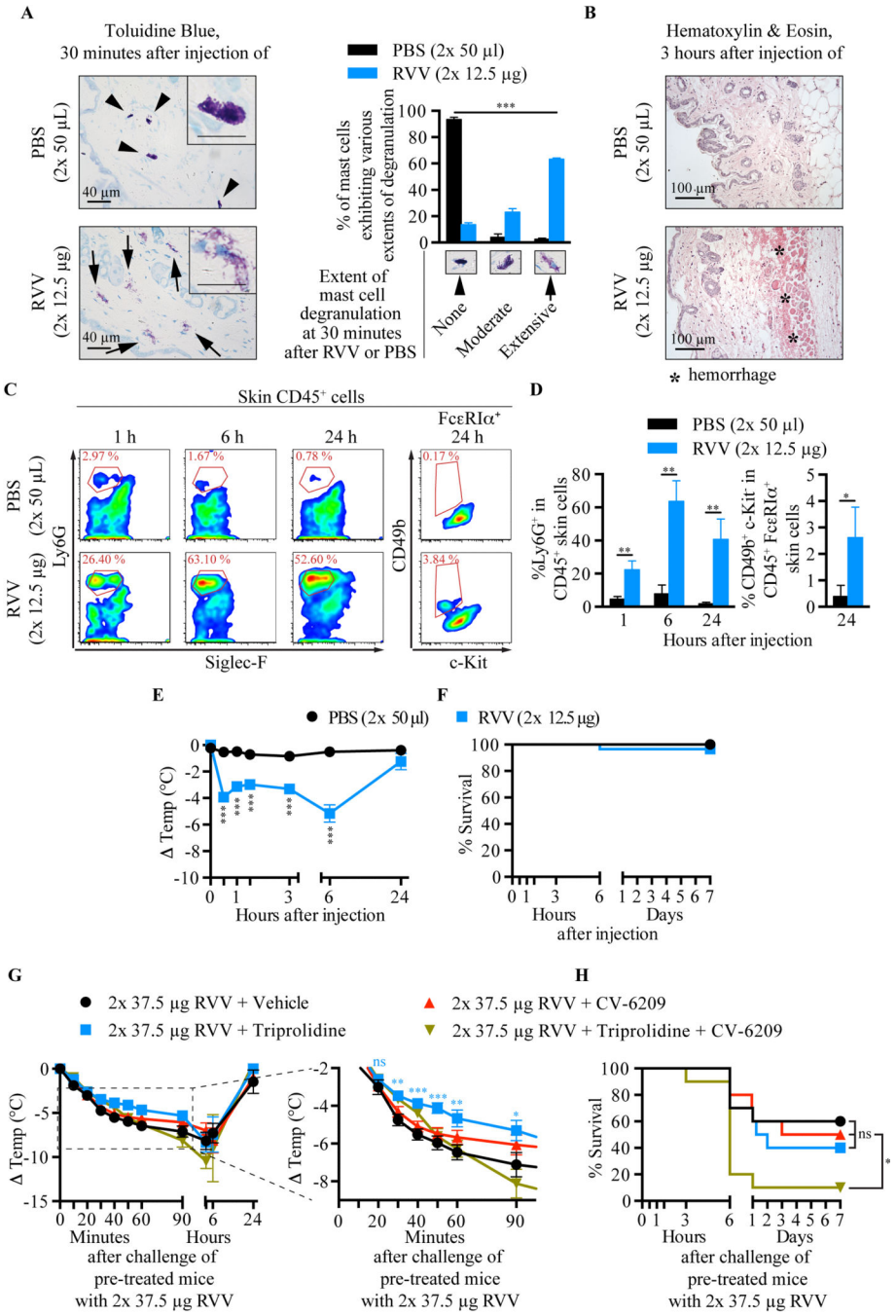


Fig 1.

RVV can induce local MC degranulation, recruitment of innate inflammatory cells, and hypothermia. **A,B**, Toluidine Blue- (*A*) and Hematoxylin & Eosin- (*B*) stained back skin sections; **A**, Extent of MC degranulation (mean+SD). **C,D**, flow cytometry plots (*C*) and quantification (*D*) (mean+SD, from 3 mice, representative of 2 experiments) of $CD45^+$ skin cells. **E**,**F**, temperature (*E*) and survival (*F*) after RVV injection. **G**,**H**, temperature (right panel magnifies the area in the dashed box) (*G*) and survival (*H*) of RVV-treated mice pretreated with anti-histamine and/or PAF-receptor antagonist. *P* values: Chi-Square test

(*A*); Student's *t* test (*D*,*E*,*G*); Mantel-Cox test (*H*). Symbols in (*E*): comparison of group in that color with vehicle-treated mice for that time point. **E**-**H**, data pooled from 2-3 experiments.

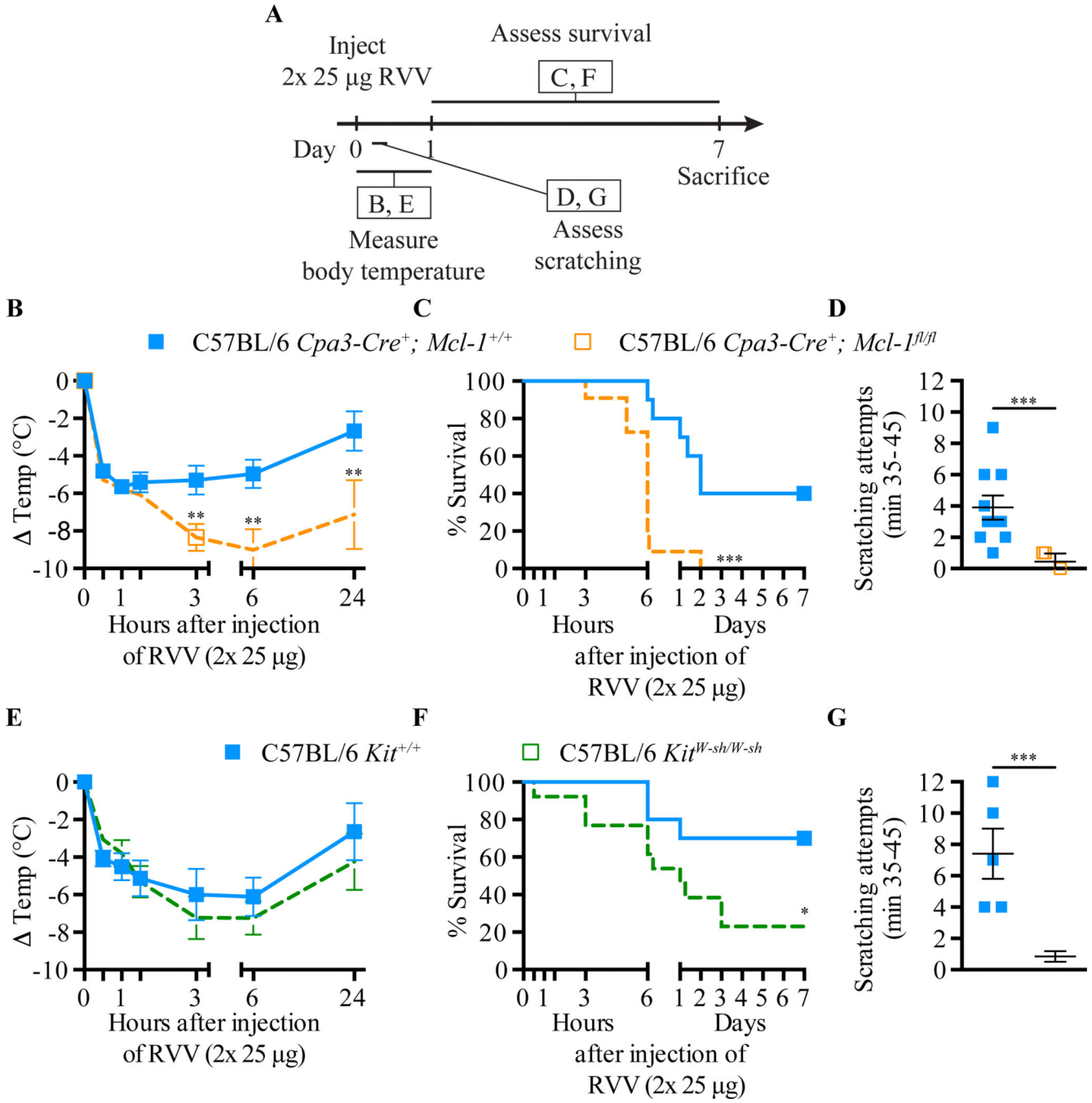


Fig 2.

MCs can contribute to innate resistance and behavioral responses to RVV. **A**, Experimental outline. **B** and **E,** body temperature; **C** and **F**, survival; **D** and **G**, scratching attempts, of MC-deficient *Cpa3-Cre*$^{+}$; *Mcl-1*$^{fl/fl}$ (*B-D*) and *Kit*$^{W\text{-}sh/W\text{-}sh}$ (*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 pooled from 2-4 experiments (n=5-21/group).

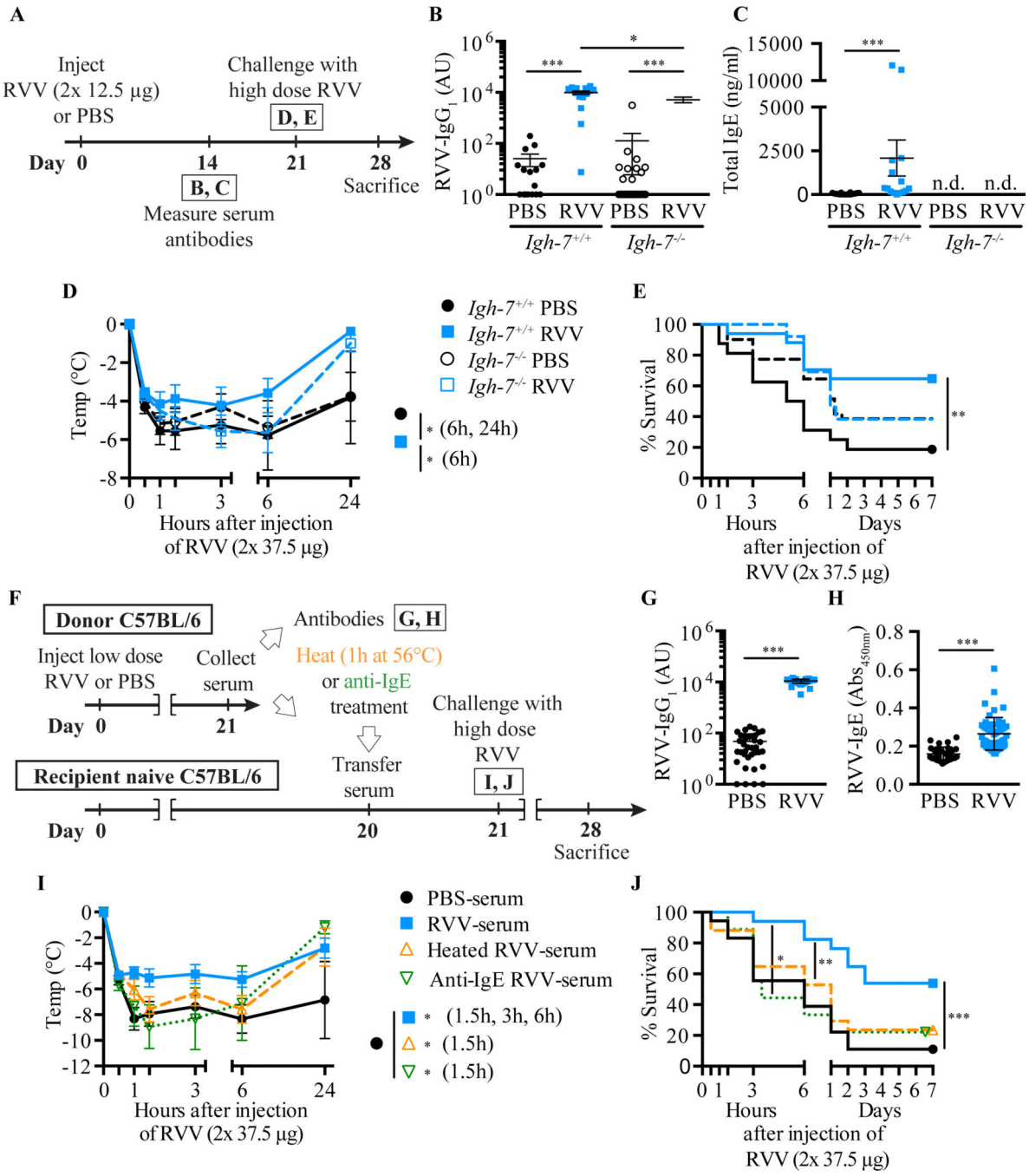


Fig 3.

IgE can contribute to acquired resistance to RVV. **A** Outline of experiments with IgE-deficient (*Igh-7*$^{-/-}$) and control (*Igh-7*$^{+/+}$) C57BL/6 mice (*B-E*). **B,C**, Serum RVV-specific IgG_1 (*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 IgG_1 (*G*) and total IgE (*H*). **I**,**J**, Body temperature (*I*) and survival (*J*). Data pooled from 3-4 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*).

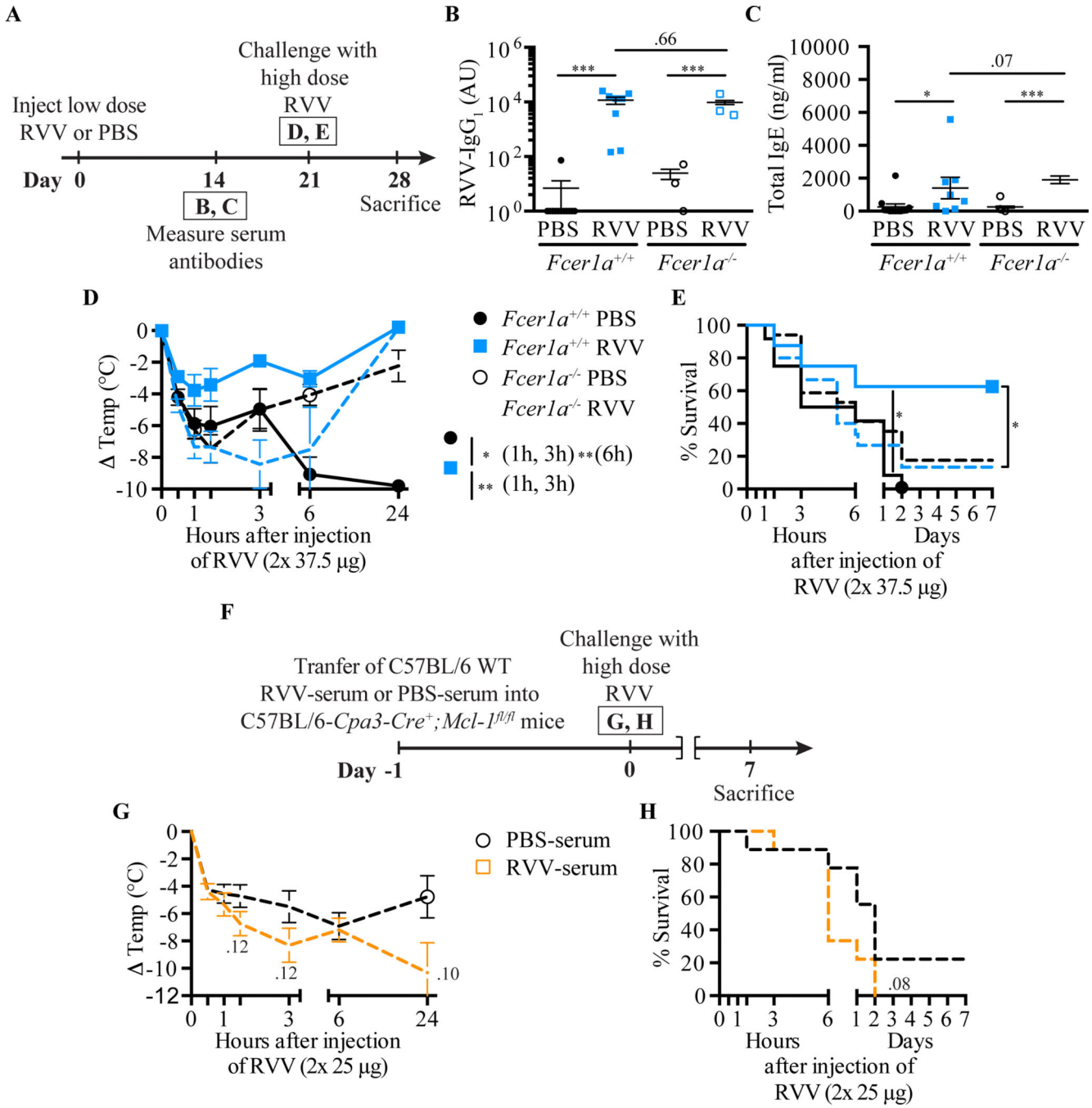
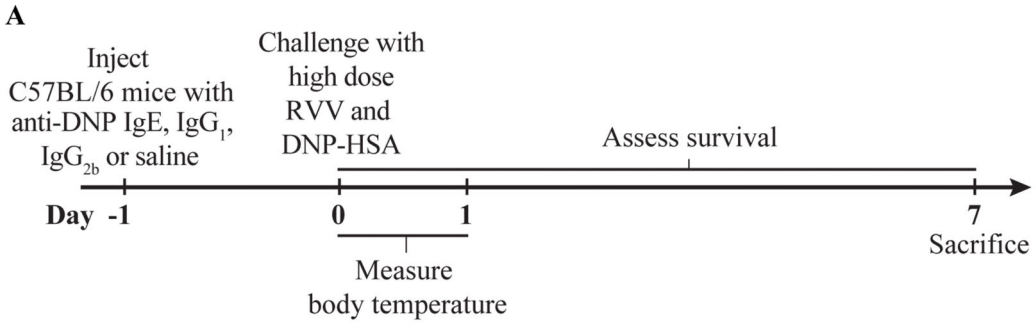
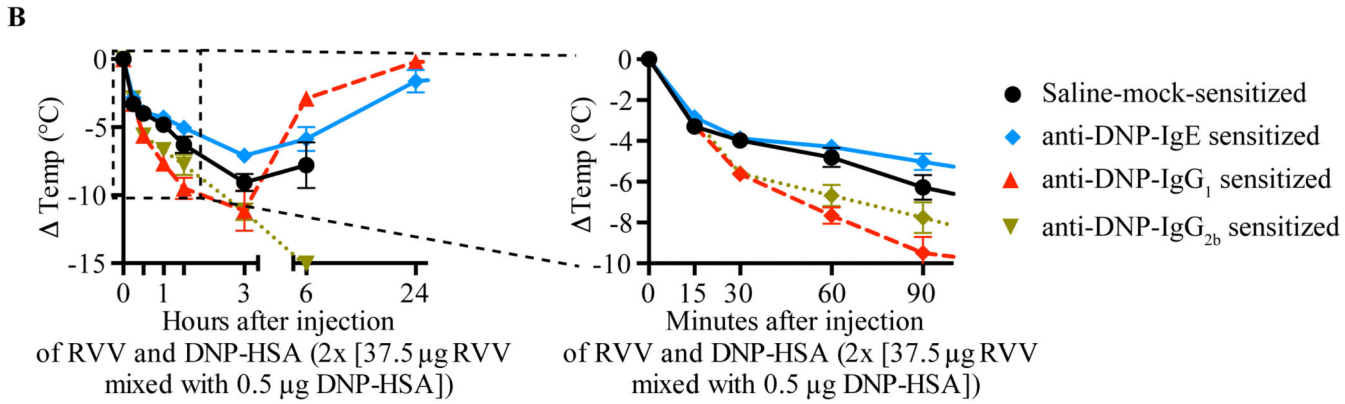


Fig 4.

FcεRIα and FcεRIα–bearing cells can contribute to acquired resistance to RVV. **A**, Outline of experiments with *Fcer1a*$^{-/-}$ and control (*Fcer1a*$^{+/+}$) C57BL/6 mice (panels B-E). **B,C**, Serum RVV-specific IgG_1 (*B*) and total IgE (*C*). **D**,**E**, Body temperature (*D*) and survival (*E*). **F**, Outline of serum transfer experiments involving MC-deficient C57BL/6 mice (*G*,*H*). **G**,**H**, Body temperature (*G*) and survival (*H*). Data pooled from 3 experiments (n=9-17/group). *P* values: Mann-Whitney test (*B*,*C*); Student's *t* test (*D*,*G*); Mantel-Cox test (*E*,*H*).





Comparisons of body temperature after injection of RVV and DNP-HSA in various groups of mice.

	Vehicle *vs.*			IgE *vs.*	
Min	IgE	IgG_1	IgG_{2b}	IgG_1	IgG_{2b}
15	*	.91	.18	*	.82
30	.76	**	**	***	***
60	.39	**	*	***	***
90	.09	**	.19	***	**
180	*	.14	.08	**	***

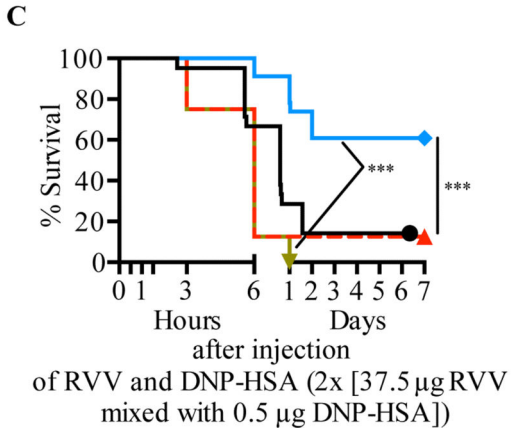


Fig 5.

IgE-dependent passive cutaneous anaphylaxis to an irrelevant antigen can increase resistance to a potentially lethal challenge with RVV. **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, IgG_1 or IgG_{2b} antibody and challenged 18 h later with 2 s.c. injections, each containing 37.5 µg RVV and 0.5 µg DNP-HSA. Data pooled from 2-5 independent experiments (n=10-25/group). *P* values: Student's *t* test (*B*); Mantel-Cox test (*C*).

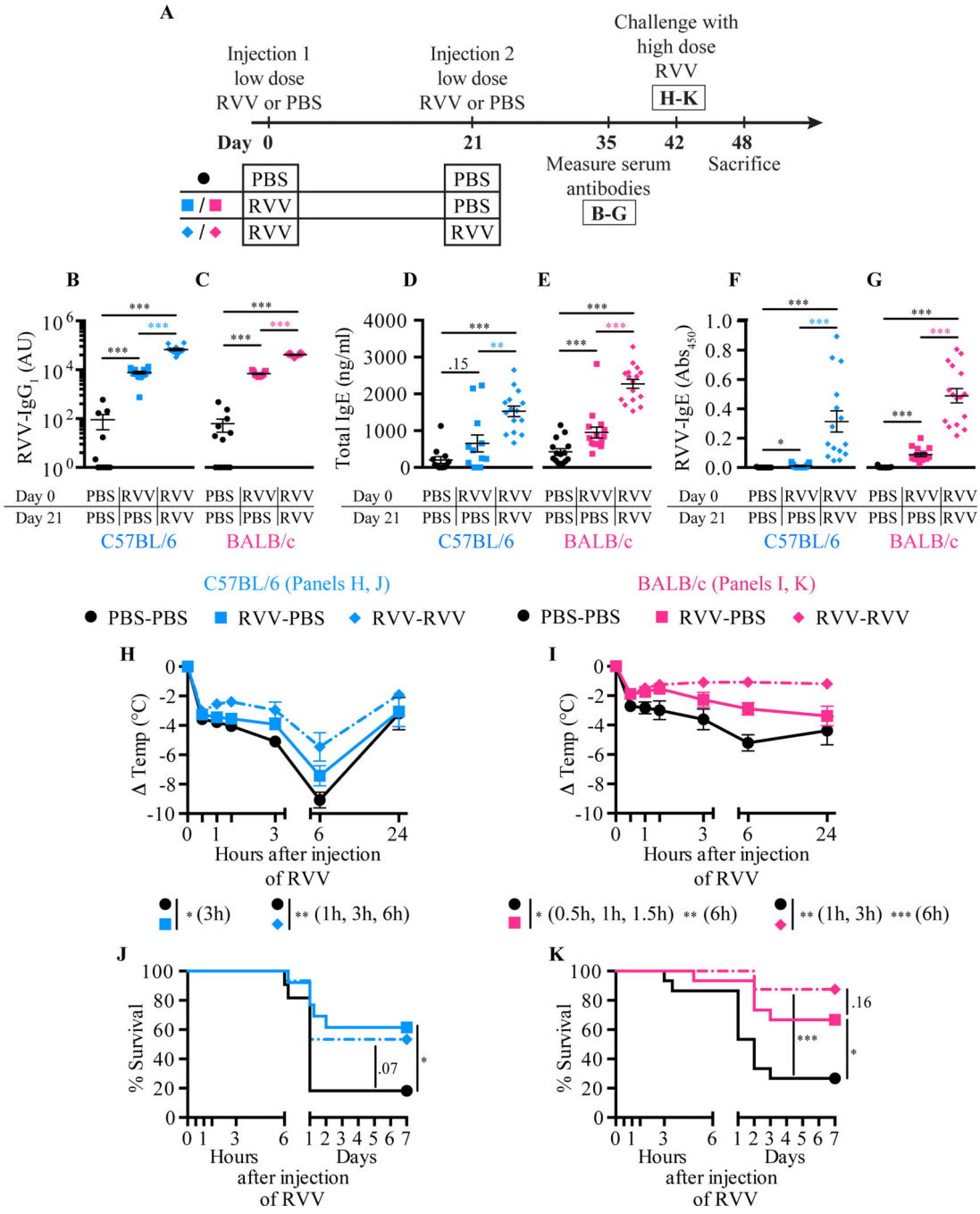


Fig 6.

Influence of genetic background and immunization regimen on acquired resistance to RVV. **A**, Experimental outline. **B-G**, Serum RVV-specific IgG_1 (*B,C*); total IgE (*D,E*); and RVV-specific IgE (*F,G*). **H,I**, Body temperature. **J,K**, Survival. Data pooled from 3-4 experiments (n=11-16/group). *P* values: Mann-Whitney test (*B-G*), Student's *t* test (*H,I*) or Mantel-Cox test (*J,K*).

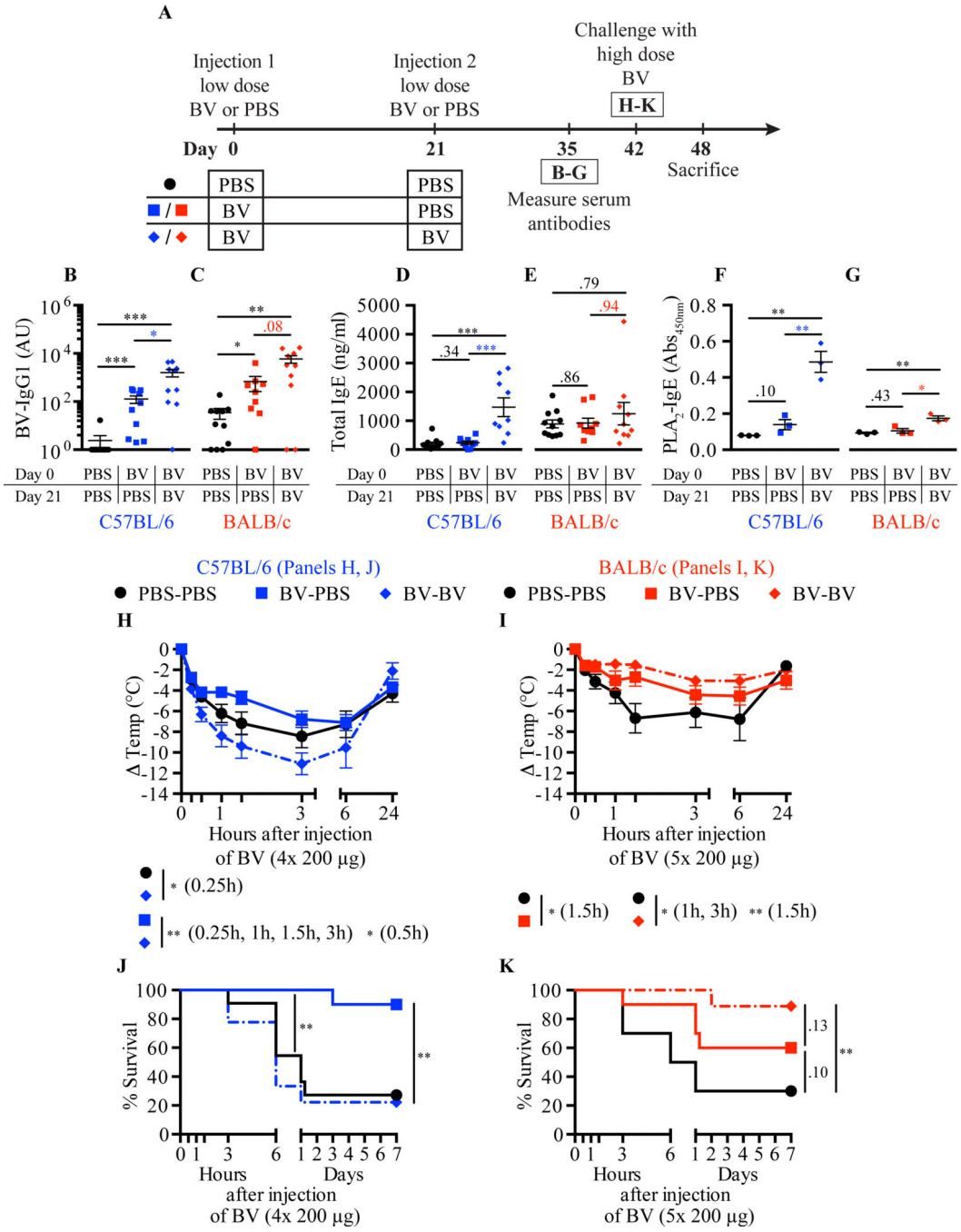


Fig 7.

Influence of genetic background and immunization regimen on acquired resistance to BV. **A**, Experimental outline. **B-G**, Serum BV-specific IgG_1 (*B*,*C*); total IgE (*D*,*E*); and $bvPLA_2$-specific IgE (*F*,*G*). **H-I**, Body temperature. **J-K**, Survival. Data pooled from 3 experiments (n=9-11/group). *P* values: Mann-Whitney test (*B-G*); Student's *t* test (*H*,*I*); Mantel-Cox test (*J*,*K*).