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Mast cell anaphylatoxin receptor expression can enhance IgE-dependent skin inflammation in mice

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Abstract

Background—Mast cells express receptors for complement anaphylatoxins C3a and C5a (i.e., C3aR and C5aR), and C3a and C5a are generated during various IgE-dependent immediate hypersensitivity reactions *in vivo*. However, it is not clear to what extent mast cell expression of C3aR or C5aR influences C3a- or C5a-induced cutaneous responses or IgE-dependent mast cell activation and passive cutaneous anaphylaxis (PCA), *in vivo*.

Objective—Assess whether mouse skin mast cell expression of C3aR or C5aR influences: 1) the cells' responsiveness to intra-dermal injections of C3a or C5a, or 2) the extent of IgE-dependent mast cell degranulation and PCA *in vivo*.

Methods—We measured the magnitude of cutaneous responses to i.d. injections of C3a or C5a, and the extent of IgE-dependent mast cell degranulation and PCA responses, in mice containing mast cells that did or did not express C3aR or C5aR.

Results—The majority of the skin swelling induced by i.d. injection of C3a or C5a required that mast cells at the site expressed C3aR or C5aR, respectively, and the extent of IgE-dependent degranulation of skin mast cells and of IgE-dependent PCA were significantly reduced when mast cells lacked either C3aR or C5aR. IgE-dependent PCA responses associated with local elevation of C3a occurred in antibody-deficient mice but not in mice deficient in FcεRIγ.

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Conflict of Interest:

The authors state no conflict of interest.

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Conclusion—Expression of C3aR and C5aR by skin mast cells contributes importantly to the ability of C3a and C5a to induce skin swelling and can enhance mast cell degranulation and inflammation during IgE-dependent PCA *in vivo*.

Keywords

Anaphylatoxin; anaphylaxis; C3a; C5a; complement; IgE; inflammation; mast cells

INTRODUCTION

Anaphylaxis is a severe acute systemic allergic reaction that can be induced when certain unfortunate subjects previously sensitized to an allergen are later exposed to even very small amounts of that allergen.^{1,2} In anaphylaxis, the offending antigen interacts with specific IgE antibodies bound to tissue mast cells and peripheral blood basophils causing them to release histamine and other mediators which induce within minutes the local and systemic clinical manifestations of this reaction.¹⁻⁴ Long ago, it was proposed that “humoral factors” derived from the incubation of normal guinea pig serum with washed immune precipitin (these factors were termed “anaphylatoxin” or “serotoxin”) may contribute to anaphylaxis.⁵ Although this hypothesis was controversial for decades,⁶ it is now known that the complement anaphylatoxins, C3a and C5a, can activate various populations of rodent⁷⁻⁹ or human⁹⁻¹⁶ mast cells *in vitro*, and that cutaneous injection of C3a or C5a can induce degranulation of rodent⁸ or human¹⁷⁻¹⁹ mast cells *in vivo*. Moreover, the complement system is activated and C3a and C5a are generated during certain immediate hypersensitivity reactions, including anaphylaxis, in humans and other mammals,²⁰⁻²² and positive correlations have been reported between the extent of complement activation, as assessed by measuring C3a levels²³ or cleavage of C3,²⁴ and the severity of systemic anaphylaxis in humans²³ or in a guinea pig model of immediate hypersensitivity.²⁴

However, despite extensive evidence associating anaphylatoxins with anaphylaxis, it isn't clear to what extent C3a or C5a responsiveness of mast cells, as opposed to other cells with anaphylatoxin receptors, contributes to C3a- or C5a-induced inflammation, or IgE-dependent immediate hypersensitivity responses, *in vivo*. Using mice containing mast cells that did or did not express the anaphylatoxin receptors C3aR or C5aR, we measured the extent to which inflammation induced by cutaneous injection of C3a or C5a reflected C3aR and C5aR expression on mast cells, and assessed the role of mast cell C3aR and C5aR in regulating the extent of IgE-dependent mast cell activation during IgE-dependent passive cutaneous anaphylaxis (PCA).

METHODS

Mice

Kit^{W-sh/+} mice from Peter Besmer (Memorial Sloan-Kettering Cancer Center) were bred to produce mast cell-deficient C57BL/6-*Kit^{W-sh/W-sh}* (*Kit^{W-sh/W-sh}*) mice and normal congenic wild-type (WT) littermates. C57BL/6J *C3aR^{-/-}* and *C5aR^{-/-}* mice were from the populations reported by Humbles et al.²⁵ and Hopken et al.,²⁶ respectively. C57BL/6 FcRγ-deficient mice (B6.129P2 *Fcer1g^{tm1Rav}* mice, backcrossed 12 generations) were from Taconic, New York, NY, USA. Antibody-deficient B6.129S2-Igh-6^{tm1Cgn}/J mice (stock number: 002288) were from the Jackson Laboratory. See Online Repository for additional details.

Anaphylatoxin-induced ear swelling

Mice under isoflurane anesthesia received an intradermal (i.d.) injection of 100 ng of C3a (Calbiochem, San Diego, CA) or C5a (R&D Systems, Minneapolis, MN) in 20 μl HMEM-Pipes buffer (500 ml Sigma M4642 (MEM) + 0.47 g Pipes + 0.105 g NaOH) in the right ear

and 20 μ l vehicle in the left ear as control. Ear thickness was measured with a dial thickness gauge (G-1A, Ozaki, Tokyo, Japan) before and at intervals after i.d. injections.

IgE-dependent passive cutaneous anaphylaxis (PCA)

Mice under isofluorane anesthesia were passively sensitized by i.d. injection of 20 ng DNP-specific IgE (α DNP clone ϵ 26,²⁷ from Dr. Fu-Tong Liu, UC Davis) in 20 μ l HMEM-Pipes buffer in the right ear; 20 μ l vehicle was injected into the left ear as control. 24 h later, mice were challenged intravenously (i.v.) with 200 μ g dinitrophenol (DNP)-conjugated human serum albumin (HSA) (Sigma) in 100 μ l 0.9% NaCl. Ear thickness was measured before and at intervals after i.v. antigen challenge. Mice were sacrificed 6 h post-challenge and ear pinnae were collected for histological analysis.

Cell culture and flow cytometry

Bone marrow-derived cultured mast cells (BMCMCs) were obtained by culturing femoral bone marrow cells from female mice in 20% WEHI-3 conditioned medium (as a source of IL-3) for 4–6 weeks, at which time >95% of the cells were identified as mast cells by May Grünwald-Giemsa (Sigma) staining and flow cytometry (Kit⁺ and Fc ϵ RI α ⁺; see Online Repository for details).

Adoptive transfer of BMCMCs into mast cell-deficient mice

BMCMCs derived from WT (C57BL/6), *C3aR*^{-/-}, *C5aR*^{-/-}, or *Fc ϵ RI α* ^{-/-} mice were transferred by i.d. injection (2 injections/ear; 1×10^6 cells in 20 μ l DMEM/injection) into 4-week old female *Kit*^{W^{sh}/W^{sh} mice 8 weeks before initiating the experiments.}

Quantification of mast cell degranulation

Mast cells were classified by histomorphometry in alkaline Giemsa-stained, plastic-embedded 1 μ m sections (see Online Repository for details).

Histological analysis of leukocytes

Mice were killed by CO₂ inhalation, and 4 μ m sections of ear pinnae fixed in 10% (v/v) buffered formalin and embedded in paraffin were stained with hematoxylin and eosin (H&E). Dermal leukocytes were counted (numbers/mm²) at a magnification of 1000X in the entire length of the ear in a blinded fashion.

Measurement of C3a

Ear skin lysates prepared by sonication of finely chopped ear pinnae (in 300 μ l T-PER EDTA-free lysis buffer [Pierce] containing protease inhibitors [Roche]) were frozen at -80°C overnight, thawed, and centrifuged at 16000 $\times$ g for 20 min at 4°C and C3a concentrations in the supernatants were measured by ELISA (see Online Repository for details).

Statistical analyses

Analysis of variance (ANOVA) was used to assess the significance of differences in ear thickness. Unless specified otherwise, differences in the numbers of dermal mast cells or neutrophils, or levels of C3a in ear lysates, were tested for significance using the 2-tailed Mann-Whitney U test. We used the Chi-square test to compare values for the extent of mast cell degranulation. See Online Repository for additional details.

RESULTS

Mast cells can enhance ear swelling responses to C3a and C5a in a complement receptor-dependent manner

Ear swelling responses after i.d. injection of C3a (Fig 1, *A*) or C5a (Fig 1, *B*) peaked at 30 min and were significantly lower in *Kit^{W-sh/W-sh}* mice compared to WT controls following the injection of either anaphylatoxin (Fig 1). *Kit^{W-sh/W-sh}* mice did not detectably respond to C3a (see Fig E1 in the Online Repository). There was a small but significant increase in the ear swelling response observed in *Kit^{W-sh/W-sh}* mice following injection with C5a versus vehicle ($P < 0.0001$), suggesting that a mast cell-independent pathway may contribute to this response. We did not observe any ear swelling responses in WT mice following injection with 1 μ g of C3 or C5 (data not shown), suggesting that these precursors need to be cleaved in order to induce a cutaneous response.

Kit^{W-sh/W-sh} mice have several abnormalities beside a profound deficiency in mast cells,²⁸⁻³¹ including elevated numbers of blood neutrophils.²⁹⁻³¹ To assess whether the adoptive transfer of mast cells to the skin of *Kit^{W-sh/W-sh}* mice would enhance their ability to respond to challenge with C3a or C5a, these mice were engrafted with WT BMCMCs. *Kit^{W-sh/W-sh}* mice engrafted with WT mast cells (WT BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice) exhibited C3a- or C5a-induced ear swelling responses similar to those observed in WT C57BL/6J mice (Fig 1). By contrast, *Kit^{W-sh/W-sh}* mice engrafted with *C3aR^{-/-}* BMCMCs (*C3aR^{-/-}* BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice) did not exhibit an ear swelling response to C3a above background (see Fig E1 in the Online Repository) or that observed in *Kit^{W-sh/W-sh}* mice (Fig 1, *A*), but exhibited responses to C5a comparable to those in WT BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice (see Fig E2, *A* in the Online Repository). Similarly, *Kit^{W-sh/W-sh}* mice engrafted with *C5aR^{-/-}* BMCMCs (*C5aR^{-/-}* BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice) did not exhibit ear swelling after injection of C5a (Fig 1, *B*), but their C3a-induced response was similar to that observed in WT BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice (see Fig E2, *B* in the Online Repository). *Kit^{W-sh/W-sh}* mice engrafted with WT, *C3aR^{-/-}* or *C5aR^{-/-}* BMCMCs had similar numbers of ear dermal mast cells, but the number in *C5aR^{-/-}* BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice was significantly lower than that in the WT BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice (see Fig E3 in the Online Repository). For all mouse groups studied, the vehicle-induced ear swelling responses were much weaker than those induced by the anaphylatoxins (see Fig E1 in the Online Repository).

Although it has been difficult to implicate antibody isotypes other than IgE in the pathogenesis of allergen-induced anaphylaxis in humans, both IgE and IgG₁ antibodies can contribute to anaphylaxis in mice.^{1,4,30,32} To determine whether C3a- or C5a-induced responses involved effects of endogenous IgE or IgG₁ antibodies mediated through Fc ϵ RI or Fc γ RIII, we assessed such responses in Fc receptor common γ -chain deficient (*Fc ϵ RI γ ^{-/-}*) mice.³³ Ear swelling responses to C3a (see Fig E4, *A* in the Online Repository) or C5a (see Fig E4, *B* in the Online Repository) in *Fc ϵ RI γ ^{-/-}* mice were comparable to those in WT controls, and significantly higher than those induced by vehicle (see Fig E4 in the Online Repository), indicating that such response can occur independently of the Fc ϵ RI γ . Moreover, in *Kit^{W-sh/W-sh}* mice engrafted with *Fc ϵ RI γ ^{-/-}* BMCMCs, the C3a- or C5a-induced ear swelling responses were similar to those in WT BMCMCs $\rightarrow$ *Kit^{W-sh/W-sh}* mice (data not shown).

IgE-dependent mast cell activation is sufficient for local C3a production during PCA reactions

We measured changes in ear thickness, skin mast cell degranulation, and levels of endogenous C3a after i.v. challenge with specific antigen in IgE-sensitized versus vehicle-

injected ear pinnae of WT C57BL/6J mice and B6.129S2-Igh-6^{tm1Cgn}/J mice, which lack all endogenous antibodies (Abs) (Fig 2). In B6.129S2-Igh-6^{tm1Cgn}/J mice, which have levels of ear pinnae (see Fig E5, *A* in the Online Repository) and peritoneal (data not shown) mast cells and peritoneal mast cell surface FcεRI and Kit levels (see Fig E5, *B* in the Online Repository) comparable to those of WT mice, IgE-dependent PCA reactions were associated with levels of ear swelling (Fig 2, *A*), skin mast cell degranulation (Fig 2, *B*), and skin C3a (Fig 2, *C*) that were even higher than those in WT mice. These results support the conclusion that C3a is produced locally during IgE-dependent PCA reactions, and that functions of native antibodies, including any role in the classical pathway of complement activation, are not required for the tissue swelling, mast cell degranulation or elevation of tissue levels of C3a at sites of IgE-dependent PCA. By contrast *FcεRIγ*^{-/-} mice failed to develop tissue swelling, enhanced mast cell degranulation or elevations in tissue levels of C3a at sites challenged to assess IgE-dependent PCA (Fig 3). As predicted, we did not observe a swelling response or mast cell degranulation in the absence of IgE (vehicle-treated ears) or FcεRIγ (Fig 3). Taken together, the data in Figs 2 and 3 indicate that IgE-dependent activation of skin mast cells via the FcεRI γ chain is sufficient to activate complement at sites of IgE-dependent PCA.

C3aR and C5aR expression by mast cells can contribute to the features of IgE-dependent PCA reactions

We found that the ear swelling associated with PCA reactions in C3aR- or C5aR-deficient mice were markedly reduced in mice lacking the C3aR or the C5aR, compared to those in WT mice (Fig 4). However, the ear swelling responses in the C5aR-deficient mice were slightly ($P < .05$) higher than those in the C3aR-deficient mice (Fig 4). We observed no significant ear swelling in the vehicle-treated ears of these mice (data not shown) and the anatomical distribution of mast cells in the ears of WT, *C3aR*^{-/-} and *C5aR*^{-/-} mice were similar (data not shown). Moreover, despite the decreased PCA responses observed in *C3aR*^{-/-} vs. WT mice, we observed slightly higher numbers of mast cells in the ears of these mice compared to WT or *C5aR*^{-/-} mice (see Fig E6 in the Online Repository). Similar levels of FcεRI and Kit were detected on peritoneal mast cells from *C3aR*^{-/-}, *C5aR*^{-/-} and WT mice (see Fig E7 in the Online Repository).

The PCA-induced ear swelling in WT BMCMCs→*Kit*^{W-sh/W-sh} mice was significantly higher than that in *Kit*^{W-sh/W-sh} mice (Fig 5, *A*) and was similar to the response observed in WT mice (Fig 4). Importantly, the BMCMCs derived from *C3aR*^{-/-} or *C5aR*^{-/-} mice that were used in our engraftment studies showed similar levels of FcεRI expression compared to WT BMCMCs by flow cytometry (data not shown). However, engraftment of *Kit*^{W-sh/W-sh} mice with *C3aR*^{-/-} or *C5aR*^{-/-} BMCMCs failed to restore the PCA-induced ear swelling response to the levels observed in WT BMCMCs→*Kit*^{W-sh/W-sh} mice (Fig 5, *A*). Similar to the results we obtained using the C3aR- and C5aR-deficient mice (Fig 4), the PCA-induced ear swelling response was greater in *C5aR*^{-/-} BMCMCs→*Kit*^{W-sh/W-sh} mice than in *C3aR*^{-/-} BMCMCs→*Kit*^{W-sh/W-sh} mice (Fig 5, *A*). These data suggest that expression of C3aR or C5aR on mast cells contributes significantly to ear swelling responses during IgE-dependent PCA reactions. We also quantified leukocytes in the dermis 6 h after inducing PCA. The reactions in ears that had been engrafted with C3aR- or C5aR-deficient mast cells contained respectively, ~50% or 60% fewer leukocytes (predominantly neutrophils and mononuclear cells) than did reactions in ears that had been engrafted with WT mast cells (Fig 5, *B*).

We also assessed the extent of mast cell degranulation in IgE-dependent PCA reactions. In WT BMCMCs→*Kit*^{W-sh/W-sh} mice, we observed extensive degranulation in ~70% of the ear dermal mast cells 6 h after challenge with antigen (Fig 5, *C*). The extent of mast cell degranulation in *Kit*^{W-sh/W-sh} mice engrafted with C3aR- or C5aR-deficient mast cells

(~35% or 15% extensively degranulated mast cells, respectively) was significantly reduced compared to that observed in WT BMCMCs→*Kit^{W-sh/W-sh}* mice (Fig 5, C). However, in all groups studied, the extent of mast cell degranulation observed at sites of IgE-dependent PCA was significantly greater than that in the contralateral vehicle-injected ears (Fig 5, C).

DISCUSSION

Our results in mast cell-engrafted *Kit^{W-sh/W-sh}* mice show that expression of anaphylatoxin receptors by the adoptively transferred mast cells can both markedly increase the skin swelling associated with i.d. injection of C3a or C5a and also markedly increase the mast cell degranulation, skin swelling, and cutaneous leukocyte infiltration associated with IgE-dependent PCA responses. Although our study focused on skin mast cells in mice, various types of rodent or human mast cells can react to C3a and/or C5a,^{7-10,12-19} C3a or C5a can stimulate human skin mast cell chemotaxis,¹⁴ as well as degranulation,^{9,10} *in vitro*, and C5a can stimulate such cells to produce the neutrophil chemotaxin interleukin-8.⁹ In addition, injection of C3a or C5a into normal mouse or human skin can result in wheal and flare reactions associated with dermal mast cell degranulation and polymorphonuclear leukocyte rich inflammatory infiltrates.^{8,17-19} Our findings in mast cell-engrafted *Kit^{W-sh/W-sh}* mice indicate that much of the local inflammation induced by the intradermal injection of C3a or C5a require that the mast cells at the site of the reaction express the appropriate anaphylatoxin receptors.

Our results also show that C3a and C5a, in contrast to bradykinin,³⁴ induce substantially less edema upon intradermal injection in *Kit^{W-sh/W-sh}* mice than in the corresponding wild type mice. We found that C3a-induced skin swelling depended almost entirely on mast cells, whereas both mast cell-dependent and mast cell-independent pathways appeared to contribute to C5a-mediated responses (i.e., some C5a-induced tissue swelling was observed in mice whose skin virtually lacked mast cells). These results are in accord with previous findings of neutrophil-rich infiltrates in mast cell-depleted human skin after i.d. challenge with C5a.¹⁹

We were particularly interested in the extent to which skin mast cell activation by anaphylatoxins reflected mast cell expression of the relevant anaphylatoxin receptors. Many studies have reported the expression of C3aR and C5aR on mast cell populations,^{11,13,35,36} and a blocking antibody against C5aR can reduce C5a-mediated degranulation of human skin mast cells *in vitro*, evidence that C5a can directly activate human skin mast cells via the C5aR.¹¹ However, other groups have reported that C3a and C5a, which are cationic, can bind non-specifically to rat peritoneal mast cells via electrostatic interactions with anionic heparin-proteoglycan on the mast cell surface.³⁷⁻³⁹ C3a- or C5a-induced ear swelling responses were significantly reduced in mast cell-engrafted *Kit^{W-sh/W-sh}* mice whose skin mast cells lacked the C3aR or C5aR, respectively, confirming that the binding of each anaphylatoxin to its specific receptors on skin mast cells can markedly enhance the skin swelling response induced by that anaphylatoxin *in vivo*.

Activation of the C5aR pathway has been shown to decrease internalization of the C3aR *in vitro* when the two anaphylatoxins are added simultaneously,⁴⁰ and C5L2, a non-signaling receptor shared by both anaphylatoxins, can positively modulate both C5a- and C3a-anaphylatoxin-induced responses.⁴¹ We found that the ear swelling response to C5a was not influenced by a lack of C3aR expression on mast cells and *vice versa* (see Fig E2 in the Online Repository), indicating that, at least under the *in vivo* conditions examined, a lack of one of the two anaphylatoxin receptors did not detectably influence the functionality of the other. Crosstalk between anaphylatoxins and Fc receptors also has been reported.^{42,43} However, we found that a general (see Fig E4 in the Online Repository) or mast cell-specific

(data not shown) deficiency in FcεRIγ did not detectably influence C3a- or C5a-mediated ear swelling responses, indicating that the cutaneous reactions induced by these anaphylatoxins are not dependent on antibody-induced signaling events, such as effects on mast cells of baseline levels of IgE that are mediated via FcεRI.⁴⁴

We especially wanted to assess whether activation of mast cells via anaphylatoxin receptors could influence the extent of mast cell degranulation, and the extent of IgE-induced tissue swelling and leukocyte infiltration, during the PCA model of IgE- and mast cell-dependent inflammation. We found that C3a was produced locally during IgE-dependent PCA (Fig 2, C). We also detected elevated levels of C5a at sites of PCA reactions in some experiments but not others (data not shown), perhaps because 2 h after antigen challenge is not the optimal time to perform such measurements. However, the mechanism by which anaphylatoxins are generated at sites of IgE-dependent PCA remains to be determined. We found no reports indicating the either mouse or human mast cells can produce C3 or C5, and detected neither of the corresponding mRNAs in WT BMCMCs by real-time PCR (see Online Repository for details). Cultured WT peritoneal mast cells exhibited only very low levels of transcript for C3 or C5, either at baseline or after stimulation with LPS or IgE and antigen (see Online Repository for details). Thus, we think it unlikely that mast cells represent a major source of C3 and C5 at sites of PCA.

Given that IgE doesn't activate complement, and our findings that tissue swelling and C3a levels at sites of IgE-dependent PCA responses actually were enhanced in Ab-deficient mice which lack IgG and IgM (Fig 2), it is unlikely that the classical pathway of complement activation is responsible for the generation of C3a (or C5a) in this model. However, the release of histamine and other vasoactive mediators from mast cells may be sufficient to generate anaphylatoxins, since both the injection of histamine into guinea pig skin⁴⁵ and IgE-dependent degranulation of cutaneous mast cells in mice⁴⁶ result in the rapid local activation of coagulation, which is known in turn to activate complement.⁴⁷ Indeed, we have confirmed that i.d. injection of histamine results in the elevation of skin levels of C3a in mice (see Fig E8 in the Online Repository).

Human β-tryptase can generate anaphylatoxins from C3, C4 and C5 at acidic pH *in vitro*, suggesting that the mast cell protease, β-tryptase, might generate complement anaphylatoxins *in vivo* at sites of inflammation.^{48,49} Although it has not yet been shown that tryptase (of human or mouse origin) can cleave complement components *in vivo*, mast cells contain a number of proteases and it is tempting to speculate that proteases (or perhaps other mediators) capable of generating anaphylatoxins are generated by activated mast cells at sites of IgE-dependent PCA. In addition to its ability to generate anaphylatoxins, it has been reported that human β-tryptase can degrade C3a^{48,49} and C4a⁴⁹ *in vitro*. Thus, it is possible that anaphylatoxins can be both generated and degraded by tryptase *in vivo*. Similarly, it has been reported that chymase (from rat peritoneal mast cells) is capable of degrading human C3a within minutes *in vitro*⁵⁰ and *in vivo*.⁵¹ However, it remains to be determined whether anaphylatoxin degradation by chymase or other mast cell-derived proteases represents a mechanism by which mast cells can regulate tissue levels of anaphylatoxins *in vivo*.

We previously showed that mast cells are required for the local tissue swelling and leukocyte (primarily neutrophil) infiltration at sites of IgE-dependent PCA in mice.⁵² In the present study, we found that expression of the anaphylatoxin receptors, C3aR or C5aR, on skin mast cells markedly enhanced IgE- and antigen-induced mast cell degranulation, ear swelling responses, and neutrophil infiltration at sites of PCA *in vivo* (Fig 5). Although our experiments focused on IgE-dependent PCA, it is possible that anaphylatoxin-dependent augmentation of mast cell function can occur in other settings that involve both complement activation and an effector role for mast cells. For example, a recent manuscript showed that

C5aR^{-/-} mice exhibited impaired neutrophil recruitment in a model of immune complex arthritis in which mast cells also have been implicated.⁹

C3aR and C5aR are expressed on antigen presenting cells and T cells, as well as on mast cells,^{53,54} basophils,⁵⁴⁻⁵⁶ and other hematopoietic cells.^{26,57} Accordingly, the mechanism(s) by which the anaphylatoxins C3a and C5a might regulate IgE-dependent allergic inflammation is of interest and has been the subject of debate. We of course recognize that there are many known or potential differences between immune responses involving IgE and/or mast cells in humans versus mice^{32,44,58}. However, the mast cell C3aR- and C5aR-mediated enhancement of IgE-dependent mast cell activation observed in our study may explain, at least in part, the positive correlation between complement activation and the severity of anaphylaxis in humans and rodent models. It may also shed light on the role of complement activation in asthma and other allergic or pathological disorders that involve mast cell activation.^{53,54,59-62}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

DNP-HSA	2,4 dinitrophenol-human serum albumin
BMCMC	bone marrow-derived cultured mast cell
MC	mast cell
PCA	passive cutaneous anaphylaxis
WT	wild-type

Key Messages

- Expression of C3aR and C5aR by skin mast cells significantly increases the ability of i.d. injections of C3a and C5a to induce skin swelling in mice.
- C3a is generated locally in the skin at sites of IgE-dependent passive cutaneous anaphylaxis in mice.
- Expression of C3aR and C5aR by skin mast cells significantly increases the ability of IgE and specific antigen to induce mast cell degranulation, skin swelling, and leukocyte infiltration at sites of IgE-dependent passive cutaneous anaphylaxis in mice.

FIG 1. Mast cells enhance C3a- or C5a- induced tissue swelling in a C3aR- or C5aR-dependent manner, respectively

Changes (Δ) in ear thickness in mice after injection (i.d.) of 100 ng C3a (A) or C5a (B). **P < .01, ***P < .001 vs. *Kit^{W-sh/W-sh}* mice, and +++ P < .001 vs. *C3aR^{-/-}* BMCMCs→*Kit^{W-sh/W-sh}* mice (A) or *C5aR^{-/-}* BMCMCs→*Kit^{W-sh/W-sh}* mice (B).

FIG 2. Ear swelling and C3a production during IgE-dependent PCA reactions are not dependent on the presence of native antibodies

(A) Changes (Δ) in ear thickness after injection (i.v.) of 200 μ g DNP-HSA into WT (C57BL/6) and Ab-deficient (B6.129S2-Igh-6^{tm1Cgn/J}) mice 24 h after i.d. injection of vehicle (left ears) or 20 ng anti-DNP IgE (right ears). *** $P < .001$ vs. corresponding vehicle control; +++ $P < .001$ vs. IgE/DNP-HSA treated WT group. (B) Percentage of mast cells (MCs) in vehicle- (V) or IgE-injected pinnae exhibiting extensive (ext), moderate (mod) or no (none) degranulation 6 h after i.v. DNP-HSA challenge. *** $P < .001$ vs. corresponding vehicle group. $P = 0.16$ for IgE/DNP-HSA treated WT vs. IgE/DNP-HSA treated Ab-deficient group. (C) C3a in ear lysates 2 h after injection (i.v.) of 200 μ g DNP-HSA into WT or Ab-deficient mice 24 h after i.d. injection of vehicle (V; left ears) or 20 ng anti-DNP IgE (right ears). *** $P < .001$ vs. vehicle; +++ $P < .001$ vs. IgE/DNP-HSA treated WT group.

FIG 3. IgE-dependent PCA reactions require the Fc receptor gamma chain

(A) Changes (Δ) in ear thickness after injection (i.v.) of 200 μ g DNP-HSA into mice 24 h after i.d. injection of vehicle (left ears) or 20 ng anti-DNP IgE (right ears). *** $P < .001$ vs. corresponding vehicle injected group; +++ $P < .001$ vs. IgE/DNP-HSA treated *Fcer1g*^{-/-} group. (B) Numbers of mast cells per mm of ear cartilage were counted in toluidine blue-stained ear pinnae sections prepared from WT or *Fcer1g*^{-/-} mice (data for vehicle- (V) or IgE-injected ears). * $P < .05$. (C) Percentage of mast cells (MCs) in vehicle- (V) or IgE-injected pinnae exhibiting extensive (ext), moderate (mod) or no (none) degranulation 6 h after i.v. DNP-HSA challenge. *** $P < .001$ vs. corresponding vehicle group, +++ $P < .001$ vs. IgE/DNP-HSA treated *Fcer1g*^{-/-} group. (D) C3a in ear lysates 2 h after injection (i.v.) of 200 μ g DNP-HSA into WT or *Fcer1g*^{-/-} mice 24 h after i.d. injection of vehicle (V; left ears) or 20 ng anti-DNP IgE (right ears). *** $P < .001$ vs. corresponding vehicle injected group; ++ $P < .001$ vs. IgE/DNP-HSA treated *Fcer1g*^{-/-} group. n.s. = not significant ($P > .05$).

FIG 4. *C3aR* and *C5aR* enhance ear swelling during IgE-dependent PCA reactions

Changes (Δ) in ear thickness after injection (i.v.) of 200 μ g DNP-HSA into mice 24 h after i.d. injection of 20 ng anti-DNP IgE (right ears). * $P < .05$, *** $P < .001$ vs. *C3aR*^{-/-} mice, and +++ $P < .001$ vs. *C5aR*^{-/-} mice.

FIG 5. Enhancement of IgE-dependent PCA reactions by mast cell expression of C3aR and C5aR

(A) Changes (Δ) in ear thickness after injection (i.v.) of 200 μ g DNP-HSA into mice 24 h after i.d. injection of vehicle (left ears) or 20 ng anti-DNP IgE (right ears). *** $P < .001$ vs. *Kit^{W-sh/W-sh}* mice; ++ $P < .01$, +++ $P < .001$ vs. *C3aR^{-/-}* BMCMCs → *Kit^{W-sh/W-sh}* mice, and ### $P < .001$ vs. *C5aR^{-/-}* BMCMCs → *Kit^{W-sh/W-sh}* mice. No swelling was detected in any of the left (vehicle-injected) ears. (B) Numbers of leukocytes in dermis of ear pinnae 6 h after i.v. DNP-HSA challenge. *** $P < .001$ for the indicated comparisons; n.s. = not significant ($P > .05$). (C) Percentage of mast cells (MCs) in vehicle- (V) or IgE-injected pinnae exhibiting extensive (ext), moderate (mod) or no (none) degranulation 6 h after i.v. DNP-HSA challenge (insets show MCs in Giemsa-stained, plastic-embedded 1 μ m sections of ear pinnae; scale bar = 10 μ m). ** $P < .01$, *** $P < .001$ vs. corresponding vehicle group, +++

$P < .001$ vs. IgE/DNP-HSA treated WT BMCMCs $\rightarrow$ *Kit*^{W-sh/W-sh} mice, # ## $P < .001$ vs. IgE/DNP-HSA treated *C3aR*^{-/-} BMCMCs $\rightarrow$ *Kit*^{W-sh/W-sh} mice.