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Evidence that the endothelin A receptor can enhance IgE-dependent anaphylaxis in mice

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Summary

Endothelin-1 has been implicated in inflammatory disorders including allergic responses. Here, we present evidence in mice that endothelin-1 may act via ET_A to increase mast cell degranulation and thus exacerbate clinical features of anaphylaxis.

Keywords

mast cell; allergy; anaphylaxis; endothelin-1; urticaria

To the Editor

Anaphylaxis is a life-threatening, systemic allergic reaction that can occur suddenly after contact with an otherwise innocuous substance. In certain individuals previously sensitized to a particular antigen, re-exposure to even very small amounts of that antigen can induce the rapid and extensive activation of mast cells (MCs) and basophils. The ensuing massive release of MC- and basophil-derived mediators can then result in severe and sometimes fatal pathophysiological responses¹. It is generally accepted that anaphylaxis in humans is mediated by activation of MCs and basophils induced by the cross-linking of FcεRI, the high affinity receptor for IgE, e.g., with IgE and specific bi- or multivalent antigen. Although IgE can also bind to the IgG receptors FcγRII and FcγRIII, and to galectin-3, which are expressed on some MC populations, IgE is thought to influence MC and basophil functions in anaphylaxis mainly through its interaction with FcεRI¹. However, it is likely that factors in addition to antigen-specific IgE can contribute to the occurrence or severity of anaphylaxis. For example, many allergic patients exhibit high levels of circulating antigen-specific IgE but never develop anaphylaxis, while other people with low concentrations of specific IgE in the blood may develop anaphylactic shock¹.

Endothelin-1 (ET-1), a 21 amino acid naturally occurring peptide with potent vasoconstrictor activity, has been reported to exacerbate certain allergic reactions in rodents.

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For example, elevated levels of ET-1 have been described in animal models of active anaphylaxis^{2, 3} and pharmacological inhibition of the endothelin receptor ET_A reportedly attenuated the magnitude of OVA-induced intestinal anaphylaxis³ or paw edema⁴ in OVA-sensitized animals. ET-1 can induce degranulation of MCs in mice^{5, 6}, suggesting one mechanism by which increased levels of ET-1 might exacerbate anaphylaxis. However, the potential interactions between MC and ET-1 in anaphylaxis are complex. For example, mouse MCs can secrete ET-1⁶, MC-derived proteases, particularly MC-derived carboxypeptidase A^{5, 7, 8}, can hydrolyze ET-1 to a biologically inactive peptide by removing the C-terminal tryptophan⁸, and pre-treatment of in vitro-derived mouse mast cells with ET-1 can significantly decrease the cells' degranulation in response to IgE and antigen in vitro⁶. In the present study, we investigated whether the severity of IgE-dependent passive systemic or cutaneous anaphylaxis is altered in mice treated with BQ-123, an antagonist of the endothelin receptor ET_A.

MCs are thought to represent a critical effector cell in IgE-dependent anaphylaxis¹. Prior functional assays and expression analyses of various MC populations demonstrated that ET_A can be expressed by connective tissue type MCs, including peritoneal MCs^{5, 6, 9}. However, immature bone marrow-derived cultured mouse MCs (BMCMCs), which have some similarities to mucosal type MCs, are largely unresponsive to ET-1 and have very low levels of receptor expression⁶. We confirmed by flow cytometry analysis the substantial expression of ET_A and weak expression of ET_B on C57BL/6 mouse peritoneal MCs (Fig 1, A), as well as the absence of detectable surface ET_B and very weak expression of ET_A on C57BL/6 mouse BMCMCs (Fig 1, B). When BMCMCs were induced to undergo further maturation by maintaining the cells in the presence of IL-4 and SCF, in addition to IL-3, the expression of ET receptors increased, to levels approaching those of freshly isolated peritoneal MCs (Fig 1, C). Taken together, these results support the notion that a robust surface expression of ET_A is a characteristic of mature mouse MCs.

Binding of ET-1 to ET_A can induce strong and rapid degranulation of MCs^{5, 6} and ET-1 levels previously have been shown to be increased in certain animal models of anaphylaxis^{2, 3}. We therefore hypothesized that ET-1 can have effects on MC-mediated allergic reactions. To investigate whether ET-1 can contribute to features of severe IgE- and MC-dependent allergic reactions, we first analyzed IgE-dependent passive systemic anaphylaxis (PSA) in mice treated with the selective ET_A antagonist BQ-123. To induce anaphylaxis, mice were sensitized intraperitoneally (i.p.) with IgE anti-DNP (in 100 μ l of sterile, pyrogen-free 0.9% NaCl [Saline]) and challenged i.p. 24 h later with 1 mg of DNP-HSA (30–40 DNP:1 HSA, Sigma) in 100 μ l of Saline. Mice were pretreated with BQ-123 (Calbiochem; 20 nmoles in 200 μ l of vehicle [Saline + 0.5% DMSO]) or 200 μ l of vehicle i.p. 45 min before challenge. Mice treated with BQ-123 exhibited a statistically significant, albeit modest, amelioration of the allergic reaction, as assessed by monitoring the ensuing change in body temperature (Fig 2, A). Furthermore, the reduced hypothermia was associated with a modest but statistically significant reduction in the extent of degranulation of peritoneal MCs, as assessed morphologically^{5, 7} (Fig 2, B), indicating that blockade of ET_A results in decreased activation of MCs in this setting. In support of this conclusion, pretreatment with BQ-123 in mice subjected to PSA also resulted in reduced levels of both mast cell-derived mouse mast cell protease-1 (mMCP-1) in the serum (Fig 2, C) and histamine in the peritoneal lavage fluid (Fig 2, D).

To assess the possible contribution of ET_A to IgE- and MC-dependent allergic responses in the skin, we next induced passive cutaneous anaphylaxis by intradermal (i.d.) injection of IgE anti-DNP (20 μ g in 100 μ l Saline) and then challenged the mice i.p. with DNP-HSA (1 mg in 100 μ l Saline) or 100 μ l of Saline alone 24 h later. The increase in ear thickness and the extent of plasma extravasation were then quantified as measures of allergic

inflammation. Injection of BQ-123 i.v. before elicitation of the allergic reaction again resulted in a significant reduction of the assessed parameters, with about 30–40% reduction in the measured responses compared to those in the identically sensitized and challenged but vehicle-treated mice (Fig 2, *E and F*).

Taken together, our findings show that treatment with the ET_A antagonist BQ-123 can ameliorate systemic or local IgE- and antigen-dependent anaphylactic responses in mice. We hypothesize that one mechanism which may contribute to this observation is that the ET_A antagonist can reduce ET_A-dependent enhancement of MC activation by endogenous ET-1 present during the allergic reaction.

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Abbreviations

MC	mast cell
BMCMC	bone marrow-derived cultured mast cell
ET	endothelin

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FIG 1.

ET_A is expressed on mature mouse MCs. Cell surface expression of ET_A and ET_B on (A) purified peritoneal MCs and on BMCMCs cultured 4 weeks in IL-3-containing medium (B) or for 2 weeks with IL-3 and then 2 weeks in IL-3, IL-4 and SCF (C). Data shown are from one of the three experiments performed, each of which gave similar results (see the Online Repository for more information about methods).

FIG 2.

Selective blockade of ET_A attenuates IgE-dependent anaphylactic reactions. (**A–D**) Body temperature (**A**), peritoneal MC degranulation (**B**), levels of mouse mast cell protease-1 (mMCP-1) in the serum (**C**), and levels of histamine in the peritoneal lavage fluid (PLF) (**D**) in C57BL/6 mice sensitized with IgE anti-DNP i.v. and then injected 24 h later with DNP-HSA or vehicle i.p. 45 min after i.p. injection of either BQ-123 or vehicle. Ear swelling (**E**) and extravasation of Evans blue (**F**) 1 h after DNP-HSA challenge in mice injected i.d. with IgE anti-DNP and challenged i.p. 24 h later with DNP-HSA (in Saline plus 1% Evans blue); mice were injected i.p. with either BQ-123 or vehicle (Veh) 10 min before DNP-HSA challenge. In (**A**), *** = $P < 0.005$ versus the corresponding IgE anti-DNP + DNP + vehicle-challenged group (ANOVA); in (**B**), * = $P < 0.05$; *** = $P < 0.005$ for the indicated comparisons (Chi-Square test); in (**C–F**), * = $P < 0.05$ versus the corresponding vehicle treated group (unpaired, two-tailed Student's *t*-test). (**A–F**) All results are presented as means $\pm$ SEMs (**A**) or means $\pm$ SEMs (**B–F**), with 8–10 mice per group in each panel (see the Online Repository for more information about methods).