

Letter to the Editor

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

To the Editor:

Anaphylaxis is a life-threatening, systemic allergic reaction that can occur suddenly after contact with an otherwise innocuous substance. In certain subjects 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 responses.¹ It is generally accepted that anaphylaxis in human subjects is mediated by activation of MCs and basophils induced by the cross-linking of FcεRI, the high-affinity receptor for IgE, for example, with IgE and specific bivalent 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 patients with anaphylaxis mainly through its interaction with FcεRI.¹ 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 have anaphylaxis, whereas other subjects with low concentrations of specific IgE in the blood can experience anaphylactic shock.¹

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. For example, increased 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 ovalbumin-induced intestinal anaphylaxis³ or paw edema⁴ in ovalbumin-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 pretreatment of *in vitro*-derived mouse MCs with ET-1 can significantly decrease the cells' degranulation in response to IgE and antigen *in vitro*.⁶ 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 anaphylaxis.¹ 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 mast cells (BMCMCs), which have some similarities to mucosal-type MCs, are largely unresponsive to ET-1 and have very low levels of receptor expression.⁶

We confirmed, using flow cytometric 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 stem cell factor, 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 murine 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

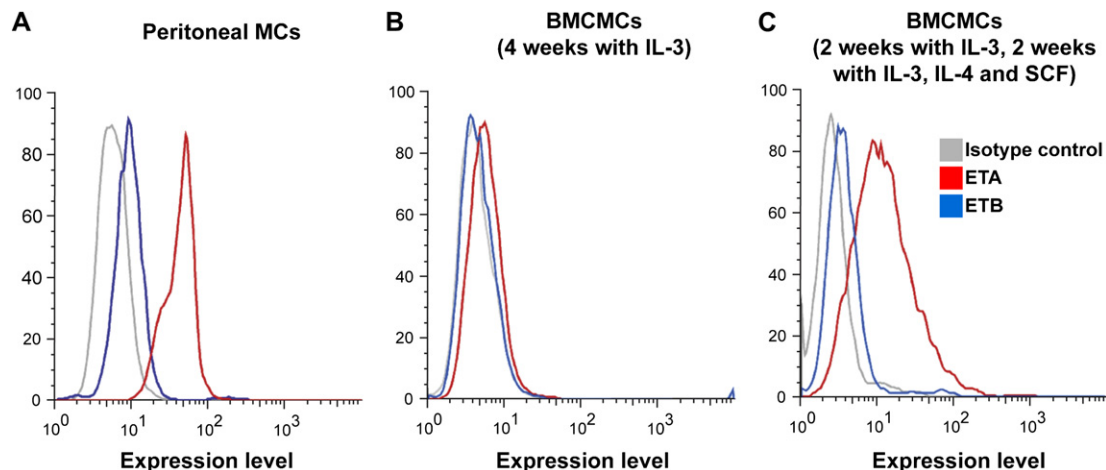


FIG 1. ET_A is expressed on mature mouse MCs. **A-C**, Cell-surface expression of ET_A and ET_B on purified peritoneal MCs (Fig 1, A) and on BMCMCs cultured for 4 weeks in IL-3-containing medium (Fig 1, B) or for 2 weeks with IL-3 and then 2 weeks in IL-3, IL-4, and stem cell factor (SCF) (Fig 1, C) is shown. Data shown are from one of the 3 experiments performed, each of which produced similar results (see the expanded Fig 1 legend in this article's Online Repository at www.jacionline.org for more information about methods).

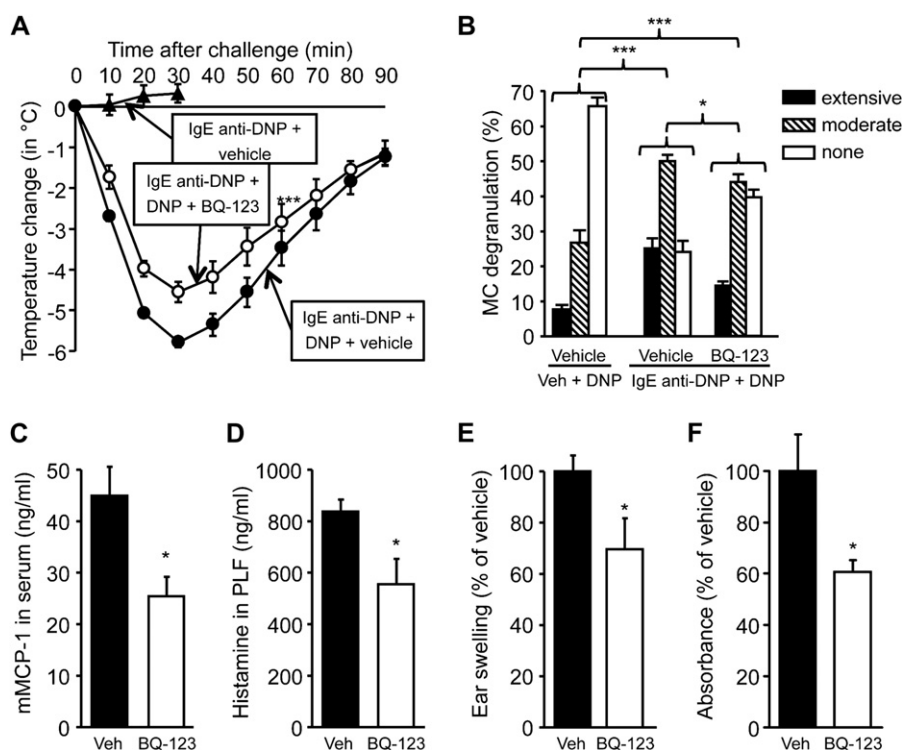


FIG 2. Selective blockade of ET_A attenuates IgE-dependent anaphylactic reactions. **A-D**, Body temperature (Fig 2, A), peritoneal MC degranulation (Fig 2, B), levels of mouse mast cell protease 1 (mMCP-1) in serum (Fig 2, C), and levels of histamine in peritoneal lavage fluid (PLF; Fig 2, D) in C57BL/6 mice sensitized with IgE anti-DNP intravenously and then injected 24 hours later with DNP-HSA or vehicle (Veh) intraperitoneally 45 minutes after intraperitoneal injection of either BQ-123 or vehicle. **E** and **F**, Ear swelling (Fig 2, E) and extravasation of Evans blue dye (Fig 2, F) 1 hour after DNP-HSA challenge in mice injected intradermally with IgE anti-DNP and challenged intraperitoneally 24 hours later with DNP-HSA (in saline plus 1% Evans blue dye). Mice were injected intraperitoneally with either BQ-123 or vehicle 10 minutes before DNP-HSA challenge. Fig 2, A, *** $P < .005$ versus the corresponding IgE anti-DNP plus DNP plus vehicle-challenged group (ANOVA). Fig 2, B, * $P < .05$ and *** $P < .005$ for the indicated comparisons (χ^2 test). Fig 2, C-F, * $P < .05$ versus the corresponding vehicle-treated group (unpaired, 2-tailed Student t test). All results are presented as means $\pm$ SEMs (Fig 2, A) or means $\pm$ SEMs (Fig 3, B-F), with 8 to 10 mice per group in each panel (see the expanded Fig 2 legend in this article's Online Repository at www.jacionline.org for more information about methods).

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 in mice treated with the selective ET_A antagonist BQ-123. Mice were sensitized intraperitoneally with IgE anti-2,4-dinitrophenol (DNP; in 100 μ L of sterile, pyrogen-free 0.9% NaCl [saline]) and challenged intraperitoneally 24 hours later with 1 mg of DNP-human serum albumin (HSA; 30-40 DNP:1 HSA; Sigma, St Louis, Mo) in 100 μ L of saline to induce anaphylaxis. Mice were pretreated with BQ-123 (Calbiochem, San Diego, Calif; 20 nmol in 200 μ L of vehicle [saline + 0.5% dimethyl sulfoxide]) or 200 μ L of vehicle intraperitoneally 45 minutes before challenge. Mice treated with BQ-123 exhibited a statistically significant, although 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 (Fig 2, B),^{5,7} 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 passive systemic anaphylaxis also resulted in reduced levels of both MC-derived mouse MC protease 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 means of intradermal injection of IgE anti-DNP (20 μ g in 100 μ L of saline) and then challenged the mice intraperitoneally with DNP-HSA (1 mg in 100 μ L of saline) or 100 μ L of saline alone 24 hours later. The increase in ear thickness and the extent of plasma extravasation were then quantified as measures of allergic inflammation. Injection of BQ-123 intravenously before elicitation of the allergic reaction again resulted in a significant reduction in the assessed parameters, with about 30% to 40% reduction in the measured responses compared with those seen 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 anaphylactic responses in mice. We hypothesize that one mechanism that might 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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APPENDIX

Expanded legend for Fig 1

Analysis of ET receptor expression on C57BL/6 mouse MCs. IL-3 in culture medium was derived from 20% WEHI-3-conditioned medium, IL-4 (from Calbiochem) was used at 30 ng/mL, and stem cell factor (from Amgen, Thousand Oaks, Calif) was used at 50 ng/mL. We assessed surface expression of ET_A and ET_B using rabbit anti-mouse ET_A and ET_B antibodies from Santa Cruz Biotechnology (Santa Cruz, Calif; M-60 and M-74, respectively).

Expanded legend for Fig 2

Elicitation and assessment of IgE-dependent anaphylactic reactions. **A**, Passive systemic anaphylaxis was induced by sensitizing C57BL/6 mice with 20 µg of IgE anti-DNP,^{E1} and mice were challenged 24 hours later intraperitoneally with 1 mg of DNP-HSA or vehicle. The selective ET_A antagonist BQ-123 (20 nmol; Bachem, Bubendorf, Switzerland) in 200 µL of vehicle (saline [sterile, pyrogen-free 0.9% NaCl plus 0.5% dimethyl sulfoxide]) or vehicle alone was injected intraperitoneally 45 minutes before DNP-HSA or saline challenge. There were 9 mice per group pooled from 3 independent experiments, each of which produced similar results. **B**, Quantitative histomorphometry was performed on peritoneal lavage-derived MCs obtained 1 hour after DNP-HSA (30-40 DNP:1 HSA; Sigma) or saline challenge, with *white bars*, *hatched bars*, and *black bars* in Fig 2, *B*, indicating no, moderate, or extensive degranulation, respectively.^{E2} There were 9 mice per group pooled from 3 independent experiments, each of which produced similar results. **C** and **D**, Levels of mouse MC protease 1 in the serum (Fig 2, *C*) were measured by means of ELISA (eBioscience, San Diego), and histamine concentrations in peritoneal lavage fluid (Fig 2, *D*) were measured with the Histareader 501 (RefLab, Copenhagen, Denmark), both according to the instructions of the manufacturer, 15 minutes after induction of passive systemic anaphylaxis. There were 9 to

10 (mouse MC protease 1) and 8 to 9 mice (histamine) per group pooled from 2 independent experiments, each of which produced similar results. Body temperature in the 2 treatment groups differed significantly 10 minutes after induction of passive systemic anaphylaxis (vehicle: $-3.3^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ vs BQ-123: $-2.5^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$; $P < .01$ unpaired Student *t* test, 2-tailed). **E** and **F**, Passive cutaneous anaphylaxis was induced in C57BL/6 mice by sensitizing them with 20 ng of IgE anti-DNP (in 20 µL of saline administered intradermally),^{E1} and control C57BL/6 mice were given 20 µL of saline administered intradermally. Mice were then challenged intraperitoneally 24 hours later with 200 µg of DNP-HSA (in 200 µL of saline plus 1% Evans blue). Mice were injected intraperitoneally with either BQ-123 (20 nmol in 200 µL administered intravenously) or vehicle 10 minutes before challenge, and ear swelling^{E3} (Fig 2, *E*) and extravasation of Evans blue dye (absorbance of the dye; Fig 2, *F*),^{E4} each calculated as the percentage of the mean result in the vehicle-treated group, were measured 1 hour after DNP-HSA or saline challenge. There were 9 mice per group pooled from 3 independent experiments, each of which produced similar results. All results are presented as means $\pm$ SEMs (Fig 2, *A*) or means + SEMs (Fig 2, *B-F*).

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