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Cutting Edge: Drebrin-Regulated Actin Dynamics Regulate IgE-Dependent Mast Cell Activation and Allergic Responses

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Mast cells play critical roles in allergic responses. Calcium signaling controls the function of these cells, and a role for actin in regulating calcium influx into cells has been suggested. We have previously identified the actin reorganizing protein Drebrin as a target of the immunosuppressant 3,5-bistrifluoromethyl pyrazole, which inhibits calcium influx into cells. In this study, we show that Drebrin−/− mice exhibit reduced IgE-mediated histamine release and passive systemic anaphylaxis, and Drebrin−/− mast cells also exhibit defects in FcεRI-mediated degranulation. Drebrin−/− mast cells exhibit defects in actin cytoskeleton organization and calcium responses downstream of the FcεRI, and agents that relieve actin reorganization rescue mast cell FcεRI-induced degranulation. Our results indicate that Drebrin regulates the actin cytoskeleton and calcium responses in mast cells, thus regulating mast cell function in vivo. The Journal of Immunology, 2015, 195: 000–000.

Stimulation via the FcεRI stimulation in mast cells results in increased intracellular Ca2+, which is critical for the function of mast cells in allergic responses. For example, mice lacking the calcium channel Orai1, or the regulator of this channel stromal interaction molecule 1 (STIM1), exhibit severely impaired histamine release and leukotriene production, reduced TNF-α secretion, and an inability to mount an anaphylactic response (1, 2). Actin cytoskeleton reorganization has been implicated in models of calcium release activated channel (CRAC) regulation. Furthermore, mast cells deficient in Wiskott–Aldrich syndrome protein, a key regulator of F-actin assembly, exhibit diminished Ca2+ mobilization, degranulation, and cytokine secretion (3). Nonetheless, little is known about actin-modulatory proteins that are involved in this process. Furthermore, given the connections between increases in intracellular calcium and actin rearrangements, it is not clear whether these defects are related to the former or the latter.

The group of immunosuppressant compounds derived from 3,5-bistrifluoromethyl pyrazole (BTP) can suppress allergy and allergic inflammation, including Ag-induced histamine release and leukotriene production in IgE-primed RBL-2H3 cells, as well as bone marrow–derived mast cells (BMMCs) in vitro and in vivo (4). We have recently determined that BTP2 potently inhibits immune cell activation via modulation of store-operated calcium channel activity, and identified the actin-binding protein drebrin 1 (Dbn1) as a target of BTP (5). We show in this study that Dbn1 regulates systemic anaphylaxis and IgE/FcεRI-induced degranulation in mast cells by regulating actin reorganization and actin dynamics.

Materials and Methods

Generation of Dbn1−/− mice

Wild-type (WT) 129S6/SvEvTac mice were obtained from Taconic, and Dbn1−/− mice on the 129/SV background were generated from a gene-trapped library of mouse ES cells (The Institute for Genomic Medicine, Houston, TX) inserted into intron 8 of the mouse Dbn1 gene (6), and backcrossed to WT 129S6/SvEvTac to generate WT littermates. Mice were genotyped by PCR using a long terminal repeat reverse primer and primer B that flanks the genomic insertion site of the gene-trap vector: long terminal repeat reverse, 5′-ATAACCCCTTGTAGTGTGAT-3′ (A), 5′-CTTC-ATCTTTGTACCTTGACACCTG-3′ (B). WT mice were littermates of the Dbn1−/− mice used for experiments, all of which were approved by the Institutional Animal Care and Use Committees at The Pennsylvania State University and Cornell University.

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Abbreviations used in this article: BMMC, bone marrow–derived mast cell; BTP, 3,5-bistrifluoromethyl pyrazole; CRAC, calcium release activated channel; Dbn1, drebrin 1; LatB, latrunculin B; SCF, stem cell factor; STIM1, stromal interaction molecule 1; WIP, Wiskott–Aldrich syndrome interacting protein; WT, wild-type.

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Generation of BMMCs

Mouse BMMCs were generated in the presence of IL-3 and or IL-3/stem cell factor (SCF) as previously described (7), and were routinely >95% positive for FcεRI and c-kit by flow cytometry. Abs were obtained from eBioscience and staining was performed in the presence of Fc block (BioLegend).

In vivo analysis of mast cells

Quantitative analysis of skin mast cells by histological staining was done as previously described (8). Transmission electron microscopy of skin samples was done as previously described (8). Analyses of peritoneal mast cells were performed by flow cytometry.

Measurements of intracellular Ca²⁺ concentration

Intracellular Ca²⁺ concentration was monitored as previously described using the Ca²⁺ indicator Fluo-4 (Invitrogen) (4). Mean calcium poststimulation was calculated as the mean of intracellular Ca²⁺ concentration Fluo-4 relative fluorescence measurements poststimulation after subtracting the mean of baseline measurements prestimulation. The slope of calcium decay was calculated as the slope of Fluo-4 relative fluorescence measurements poststimulation fitted by linear regression analysis after subtracting the mean of respective prestimulation baseline measurements.

Western blotting and analysis of mRNA

Lysates from neocortical homogenates of 6- to 10-wk-old mice were analyzed by SDS-PAGE and Western blotting as previously described (7). Analysis of mRNA was performed as previously described using TaqMan gene expression assay probes for Dbn1 exons 8–10, with GAPDH as a housekeeping gene and WT set as 1 (Applied Biosystems, Branchburg, NJ) (7).

In vivo histamine release, passive systemic anaphylaxis, and degranulation assays

Analysis of degranulation in vitro and of in vivo histamine release was determined as previously described (7). Passive systemic anaphylaxis was done as previously described (4). Mice were treated with BTP2 i.p. (25 mg/kg) or vehicle (DMSO/ethanol) 1 h before i.v. injection with 8–10 μg anti-IgE. Dbn1⁻/⁻ mice were only injected with anti-IgE (10 μg). Mice were then monitored for temperature using a rectal temperature probe.

Results and Discussion

Dbn1-deficient mice have reduced numbers of skin and peritoneal mast cells, and exhibit reduced histamine release and passive systemic anaphylaxis

We have previously reported that the actin-binding protein Dbn1 is a target of BTP2, an immunosuppressant that can inhibit mast cell degranulation and cytokine secretion (4, 5). To examine the role of Dbn1 in mast cell function, we generated Dbn1⁻/⁻ mice (Supplemental Fig. 1A). Dbn1⁻/⁻ skin tissue-resident mast cells had normal phenotype; however, there was a significant reduction in their density, and in the number of mast cells in the peritoneum, although there was no difference in mast cell density in intestine, trachea, or tongue (Supplemental Fig. 1B–E). We also found that Dbn1⁻/⁻ mice had significantly lower levels of histamine in the serum compared with WT mice during acute IgE challenge in passive systemic anaphylaxis (Fig. 1A). Furthermore, Dbn1⁻/⁻ mice exhibited reduced hypothermia during passive systemic anaphylaxis similar to mice treated with BTP2 (Fig. 1B).

Dbn1 regulates FcεRI-mediated degranulation and intracellular calcium mobilization downstream of FcεRI in BMMCs

We generated BMMCs in vitro to examine whether the reduced degranulation observed in vivo was intrinsic to mast cells. We found that there was no significant difference in differentiation of precursors into c-Kit/FcεRI double-positive mast cells over the 6-wk period in the presence of either IL-3 or IL-3 plus SCF (Fig. 1D), although Dbn1⁻/⁻ exhibited reduced proliferation in response to IL-3, which was rescued by the inclusion of SCF (Fig. 1C).
Dbn1<sup>−/−</sup> BMMCs exhibited reduced degranulation upon FceRI stimulation with IgE/Ag (Fig. 1E). This difference was not due to surface levels of FceRI (Supplemental Fig. 2A) or intracellular histamine levels (data not shown). There was no difference in global patterns of tyrosine phosphorylation, or activation of ERK, JNK, or p38 MAPK pathways in BMMCs challenged with Ag/IgE/FceRI (Supplemental Fig. 2B, 2C).

However, whereas WT BMMCs exhibit FceRI-induced stable and prolonged increase in intracellular Ca<sup>2+</sup>, which includes both release from intracellular stores and influx from outside the cells (indicated by a positive slope), Dbn1<sup>−/−</sup> BMMCs were defective in this response as indicated by a negative slope, as well as a lower mean increase in intracellular Ca<sup>2+</sup> (Fig. 2A–C). There was no difference in tyrosine phosphor-

**FIGURE 3.** Dbn1<sup>−/−</sup> BMMCs have altered actin superstructure. (A) WT (solid lines) and Dbn1<sup>−/−</sup> (dotted lines) BMMCs were stained with Alexa Flour–tagged phalloidin and analyzed by flow cytometry. (B) Resting WT (left panels) and Dbn1<sup>−/−</sup> (right panels) BMMCs were stained with Alexa Flour–tagged phalloidin and counterstained with DAPI, then analyzed by confocal microscopy. Scale bar, 10 μM. (C) WT and Dbn1<sup>−/−</sup> BMMCs were armed with anti-DNP IgE, then stimulated with DNP–human serum albumin for the indicated time, followed by analysis of F-actin using Alexa Flour–tagged phalloidin and flow cytometry. (D) Line-scan analysis of cells plotted as a function of cell width (25–35 cells each) and a function of the indicated time after activation with IgE/FceR. Left panels, WT. Middle panels, Dbn1<sup>−/−</sup>. Right panels, Statistical analysis (−log<sub>10</sub>p shown) comparing WT and Dbn1<sup>−/−</sup> actin localization across the cell. Signals >1.3 are statistically significant (p < 0.05). (E) Statistical analysis (−log<sub>10</sub>p shown) comparing difference in actin location as a function of the indicated time after activation with IgE/FceR. Left panels, WT. Right panels, Dbn1<sup>−/−</sup>. Signals >1.3 are statistically significant (p < 0.05). (F) Area under the curve (AUC) collected for the statistical analysis shown in (E). Time 0 set at 0.

![image](http://www.jimmunol.org/Downloadedfrom/)
ylation of PLCγ1, which regulates Ca^{2+}, indicating that the defect in Ca^{2+} mobilization is unlikely to be due to defects in activation of PLCγ1 (Supplemental Fig. 2D). Further analysis of the effects of these differences in Dbn1^{−/−} BMMCs revealed that Dbn1^{−/−} BMMCs secreted significantly reduced IL-2 and GM-CSF, intracellular calcium-dependent cytokines (9), but not IL-6, after FcεRI triggering compared with WT BMMCs (Supplemental Fig. 2G).

Dbn1 regulates actin organization in BMMCs

Dbn1 is frequently found to be associated with actin in the dendritic spines of neurons, and has been shown to interact with and induce changes in the actin cytoskeleton when overexpressed (10, 11). Actin has been suggested to play a role in the regulation of Ca^{2+} influx into cells (12–15), and we found that Dbn1^{−/−} BMMCs have higher levels of F-actin (detected by fluorescently tagged phalloidin) than WT cells (Fig. 3A, 3B). Furthermore, although this F-actin was found to be concentrated just under the plasma membrane of resting WT BMMCs, in the absence of Dbn1, it was more distributed inside the cell (Fig. 3B). There was also a statistically significant increase in intracellular F-actin in the absence of Dbn1 (Fig. 3B, 3D). IgE/FcεRI stimulation of these BMMCs resulted in reduced F-actin in WT cells as previously reported (16), but there was a delay in this change in Dbn1^{−/−} BMMCs (Fig. 3C).

Analysis of the distribution of F-actin in WT and Dbn1^{−/−} BMMCs at rest and after Ag/IgE/FcεRI triggering revealed WT BMMCs had F-actin primarily, if not exclusively, localized under the regions of the plasma membrane of the cells as indicated by the signal at the edges of the cells (Fig. 3B, 3D, left panels). However, this was significantly different in Dbn1^{−/−} BMMCs, which had higher levels of F-actin on the inside of the cell as well (Fig. 3B, 3D, compare left and middle panels, with statistical comparison in the right panels). IgE/FcεRI triggering resulted in significant but low-level reorganization of F-actin in WT BMMCs, primarily inside the cell (and not at the plasma membrane) over the time frame of analysis (statistical comparison with time 0; Fig. 3D, 3E). By contrast, IgE/FcεRI triggering of Dbn1^{−/−} BMMCs resulted in large-scale changes in F-actin structure inside the cell, with changes both inside the cell and at regions near the plasma membrane at 5 min, which transitioned to changes localized near the plasma membrane at 10 and 30 min (statistical comparison time 0; Fig. 3D, 3E). Comparison of the area under the curve as a crude measure of the statistically significant changes revealed that Dbn1^{−/−} BMMCs exhibit more significant changes in F-actin superstructure after IgE/FcεRI triggering compared with WT BMMCs (Fig. 3F). Note that the absence of Dbn1 did not affect FcεRI-induced phosphorylation of p21-activated kinase, suggesting that p21-activated kinase is not affected by this pathway (Supplemental Fig. 2F).

To determine whether altering the dynamics of the F-actin superstructure in these cells would rescue the behavior of the Dbn1 mutant cells, we treated Dbn1^{−/−} BMMCs with varying concentrations of latrunculin B (LatB), which interacts with F-actin and induces depolymerization. Note that at these concentrations, F-actin is still present in the cells, unlike the case at higher concentrations (Supplemental Fig. 2E). We found that LatB induces a reduction in F-actin in Dbn1^{−/−} cells (Supplemental Fig. 2E) and rescues FcεRI-induced degranulation, as well as enhances the response of WT cells in vitro in response to Ag cross-linking (Fig. 4). These data indicate that Dbn1 regulates mast cell function by regulating the dynamics and/or location of F-actin in BMMCs, which is required for efficient mast cell responses to FcεRI triggering.

Our previous identification of Dbn1 as a target for the immunosuppressant BTP, a known regulator of intracellular Ca^{2+} influx into cells (5), along with this work supporting a role for Dbn1 in regulating actin reorganization, Ca^{2+} influx, and mast cell function, adds support to the idea that the actin cytoskeleton plays an important role in regulating this process. We and others have previously reported evidence supporting a role for actin and cytoskeletal rearrangement in the regulation of Ca^{2+} influx into cells (17–19). In B cells, T cells, and mast cells, low concentrations of LatB are able to enhance cell activation and increase in intracellular Ca^{2+} (12, 13, 17). The actin regulators Wiskott–Aldrich syndrome protein, Wiskott–Aldrich syndrome interacting protein (WIP), and WAVE2 have been demonstrated to regulate store-operated channel activity and Ca^{2+} influx into mast cells and/or T cell, and in the case of WIP, regulate actin changes upon activation (3, 12, 20). Our work suggests that similar to the case with WIP, Dbn1 regulates actin changes downstream of the FcεRI. The ability to modulate the actin superstructure and calcium responses links Dbn1 to the FcεRI response leading to degranulation by mast cells. Altogether, our data support a model where influx of Ca^{2+} is regulated by modulating actin cytoskeleton in part via Dbn1, leading to mast cell activation and degranulation. Our data validate Dbn1 as a potential target for mast cell–regulated diseases.

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Disclosures

The authors have no financial conflicts of interest.

![Figure 4.](https://example.com/figure4.png)
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