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Role of Galectin-3 in Mast Cell Functions: Galectin-3-Deficient Mast Cells Exhibit Impaired Mediator Release and Defective JNK Expression

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Galectin-3 is a member of the β-galactoside-binding animal lectin family expressed in various cell types, including mast cells. To determine the role of galectin-3 in the function of mast cells, we studied bone marrow-derived mast cells (BMMC) from wild-type (gal3+/+) and galectin-3-deficient (gal3−/−) mice. Cells from the two genotypes showed comparable expression of IgE receptor and c-Kit. However, upon activation by FcεRI cross-linkage, gal3−/− BMMC secreted a significantly lower amount of histamine as well as the cytokine IL-4, compared with gal3+/+ BMMC. In addition, we found significantly reduced passive cutaneous anaphylaxis reactions in gal3−/− mice compared with gal3+/+ mice. These results indicate that there is a defect in the response of mast cells in gal3−/− mice. Unexpectedly, we found that gal3−/− BMMC contained a dramatically lower basal level of JNK1 protein compared with gal3+/+ BMMC, which is probably responsible for the lower IL-4 production. The decreased JNK1 level in gal3−/− BMMC is accompanied by a lower JNK1 mRNA level, suggesting that galectin-3 regulates the transcription of the JNK gene or processing of its RNA. All together, these results point to an important role of galectin-3 in mast cell biology.

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The mice were sacrificed and the ears were cut into several pieces to facilitate extraction of the dye. The tissue from each ear was placed in 300 μl of 0.5% Na2SO4 and 700 μl of acetone overnight at room temperature. Then, 150-μl samples were transferred into a flat-bottom microtiter plate and the absorbance at 620 nm was read in an automated ELISA plate reader. There was little or no extravasation of the dye in control ear, but the control contralateral absorbance value (± 0.05) was subtracted from the experimental value for each pair of ears from a mouse. The values were converted to concentrations by using serially diluted (0.312–20 μg/ml) solutions of Evans blue in saline as standards.

**Immunoblot analysis**

Immunoblot analyses of galectin-3 (28) and kinases (29) were performed as described. The primary Abs used were: JNK and JNK1/2, mouse mAb recognizing, respectively, JNK1 only and both JNK1 and JNK2 (BD Pharmingen); ERK1/2 and p38, respective mouse mAb (Santa Cruz Biotechnology); galectin-3, rabbit polyclonal Ab to human galectin-3 (28); tunicamycin, a mouse mAb (Sigma-Aldrich); phosphorylated c-Jun (p-c-Jun) rabbit antibody-p-c-Jun Ab (Cell Signaling Technology); phosphorylated c-Jun for activation of T cells (LAT) and phospholipase C (PLC) γ1, respective rabbit Ab (Cell Signaling Technology); LAT, PLCγ1, and PLCγ2, respective rabbit Ab (Santa Cruz Biotechnology). Secondary Abs used were: HRP-conjugated goat anti-mouse IgG and HRP-conjugated goat anti-rabbit IgG (Chemicon International).

**In vitro kinase assay**

IgE-sensitized BMMC were stimulated with 100 ng/ml DNP-BSA in Tyrode buffer for indicated time periods at 37°C. The determination of the JNK activity of the cell lysates in vitro was performed using GST-c-Jun (1–79) as the substrate, as described (29).

**Measurement of protein kinase C (PKC) activation associated with granule exocytosis**

IgE-sensitized BMMC were stimulated with 30 ng/ml DNP-BSA for indicated time periods. The cells were extracted with lysis buffer (1% Nonidet P-40, 25 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 100 mM NaVO₃, and 1000× diluted protease inhibitor mixture (Sigma-Aldrich)) on ice for 30 min. A total of 100 μg of total lysate protein were mixed with 1 μg of anti-PKCζ Ab (Santa Cruz Biotechnology) followed by 20 μl of protein G-Sepharose 4B beads (Zymed Laboratories). After the beads were washed with lysis buffer, the bound proteins were eluted and analyzed by immunoblotting using anti-p-Pan-PKC (Cell Signaling Technology) and anti-PKCζI. The relative density of the p-PKCζI over total PKCζI protein bands was analyzed by using ImageJ version 1.36 (W. S. Rasband, National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/) and ImageJ (2006).

**Measurement of calcium influx**

IgE-sensitized BMMC were resuspended in 50 μl of culture medium and mixed with 50 μl of Ca²⁺ detecting dye (final concentration of Fluo-4: 1 μg/ml, Fura Red: 2 μg/ml, probenecid: 25 μM, and Pluronic F-127: 0.02%) in Tyrode buffer. The mixtures were incubated at room temperature for 15 min. The cells were stimulated with 30 ng/ml or 1 μg/ml DNP-BSA and analyzed on a Coulter EPICS XL flow cytometer (Coulter). List mode files were analyzed using FlowJo (Tree Star). Fura Red, Fluo-4, and Pluronic F-127 are all from Molecular Probes. Probenecid was from Alfa Aesar.

**Polymerase chain reactions**

First, quantification of JNK mRNA by RT-PCR was performed. Total RNA from resting and activated BMMC was isolated using TRI reagent (Molecular Research Center) following the manufacturer’s protocol. To synthesize the first strand cDNA, 5 μg of total RNA was reverse transcribed in a 25-μl reaction using SuperScript II preamplification system (Invitrogen Life Technologies). With 1 μl of the reaction product as a template, JNK1 cDNA was amplified by PCR using forward (ctgaaggatcataccagacg) and reverse primers (ctgaaggatcataccagacg). As a control, G3PDH cDNA was amplified by using Omniscript reverse transcriptase kit (Qiagen) according to the manufacturer’s protocol. A total of 1 μg of total RNAs was used to synthesize cDNA by using Omniscript reverse transcriptase kit (Qiagen) according to the manufacturer’s instructions. Real-time PCR was performed using the TaqMan probes for mouse IL-4 and GAPDH (30) and the iCycler.
iQ system (Bio-Rad).

**JNK promoter reporter assay**

The promoter region of the human Jnk gene 1 kbp upstream of the transcription start site was cloned into pGL 3 vector (Promega), resulting in pGLJNK1-Luc. gal3<sup>−/−</sup> BMMC (5 × 10<sup>5</sup>) were transfected with 20 μg of a plasmid containing galectin-3 cDNA (pEF1gal3) (31) or the control plasmid pEF1, in combination with 6 μg luciferase reporter vector (phNK1-Luc) and 4 μg of control vector (pCMV-LacZ) by electroporation (Bio-Rad electroporator; 1000 μF and 240V). The cells were cultured in 2 ml of complete medium for 48 h and then harvested for luciferase activity assay. Luciferase values (relative light units) were calculated by dividing the luciferase activity by the β-gal activity.

**Statistical analysis**

Statistical analysis of control and experimental groups was accomplished by Student’s t test or ANOVA using the software Statview 4.01. Values of p < 0.05 were considered significant.

**Results**

**gal3<sup>−/−</sup> BMMC exhibit diminished degranulation**

To investigate the role for galectin-3 in mast cell activation, we compared BMMC from gal3<sup>−/−</sup> and gal3<sup>+/+</sup> mice with regard to their response to FceRI cross-linkage. BMMC were first sensitized with anti-DNP IgE and then stimulated with a multivalent Ag, DNP-BSA, and histamine release was measured. As shown in Fig. 1A, BMMC from gal3<sup>−/−</sup> mice exhibited significantly lower histamine release over a wide range of Ag concentrations used. In these experiments, we found that BMMC from the two genotypes contained comparable amounts of histamine (data not shown).

To exclude the possibility that galectin-3 deficiency results in aberrant FceRI expression, we measured the basal level expression of FceRI on the cell surfaces of both genotypes by flow cytometry and did not find any difference (Fig. 1B). FceRI on mast cells is known to be induced by IgE. We treated the cells with increasing concentrations of IgE and then measured the levels of cell surface FceRI. gal3<sup>−/−</sup> and gal3<sup>+/+</sup> BMMC displayed comparable amounts of FceRI after treatment with various doses of IgE (data not shown). We also determined the levels of another marker of mast cell differentiation, c-Kit, which is the receptor for SCF and plays a key role in mast cell proliferation and differentiation. The expression of c-Kit in the two genotypes was comparable (Fig. 1C).

**gal3<sup>−/−</sup> BMMC exhibit diminished cytokine production**

Besides degranulation, mast cell activation also leads to signaling events resulting in production of various cytokines, including IL-4, gal3<sup>−/−</sup> and gal3<sup>+/+</sup> BMMC were activated by FceRI cross-linkage with various doses of DNP-BSA and IL-4 in the culture supernatants was quantitated by ELISA. As shown in Fig. 2A, gal3<sup>−/−</sup> BMMC produced greater amounts of IL-4 than gal3<sup>+/+</sup> BMMC at all of the doses of DNP-BSA used. The time course of the response of the two genotypes to DNP-BSA was also compared and the reduced IL-4 release from gal3<sup>−/−</sup> BMMC was apparent at all the time points tested (Fig. 2B). Thus, the reduced response of gal3<sup>−/−</sup> BMMC was not due to slower kinetics.
The above experiments required the sensitization of the cells with IgE overnight and there existed a possibility that the defect observed for gal3⁻/⁻ BMMC was due to an altered response in the sensitization phase. We performed experiments in which BMMC were cultured in plastic wells coated with anti-DNP IgE, and thus activated directly, as cell surface FcεRI was cross-linked by solid phase IgE. As shown in Fig. 2C, IL-4 production was significantly lower in gal3⁻/⁻ BMMC compared with gal3⁺/⁺ BMMC.

gal3⁻/⁻ mice exhibit reduced PCA reactions
To determine whether galectin-3 deficiency results in a defective mast cell response in vivo, we compared the PCA reactions in gal3⁺/⁺ and gal3⁻/⁻ mice. Mice were given serial dilutions of mouse monoclonal anti-DNP IgE intradermally and then challenged with the multivalent Ag DNP-BSA i.v. The cutaneous reaction was gauged by the extravasation of the i.v. injected dye at the sensitized sites. As shown in Fig. 3, gal3⁻/⁻ mice showed significantly lower cutaneous reactions compared with gal3⁺/⁺ mice. Mast cell numbers in the skin were not significantly different between the two genotypes (data not shown).

gal3⁻/⁻ BMMC are defective in the expression of JNK1 protein
It has been shown that recombinant galectin-3 can induce mediator release from mast cells, when added to cultures of these cells (17). Thus, one possible cause for the difference between gal3⁺/⁺ and gal3⁻/⁻ BMMC in response to activation is that galectin-3 released by the cells might activate the neighboring cells in a paracrine fashion. We performed the activation of BMMC in the presence of lactose, which is known to inhibit the binding of galectin-3 to glycoconjugates (17, 32). The amount of IL-4 released was not affected by lactose in both genotypes of mast cells (data not shown).

Galectin-3 has been shown to be present inside mast cells (16) and have a number of different intracellular functions in various other cell types (reviewed in Ref. 8). Thus, galectin-3 may regulate the mast cell response through an intracellular mechanism(s). Because our results show that galectin-3 is involved in IL-4 production, we focused on the signal transduction pathway leading to transcriptional activation of the cytokine gene in mast cells. We found that gal3⁺/⁺ and gal3⁻/⁻ BMMC did not differ in the phosphorylation of ERK1/2 and MEK (data not shown). However, when we analyzed activation of JNK, we found a strikingly diminished basal protein level of the 46-kDa isoform of JNK1 in gal3⁻/⁻ BMMC (Fig. 4A). There is no difference in the 55-kDa isoform of this kinase (Fig. 4A). When the same membrane was reprobed with Ab that recognizes both JNK1 and JNK2, there was no appreciable difference in the signals from mast cells between the two genotypes. The results suggest that the Ab may detect primarily JNK2 and thus the difference in JNK1 was masked.

The decreased JNK1 protein level in gal3⁻/⁻ BMMC is not due to a global defect in protein expression in these cells. The expression levels of different kinases, as well as galectin-3 and tubulin in the cell lysates of gal3⁺/⁺ and gal3⁻/⁻ cells, were determined by immunoblotting using specific Abs. A and B, Unstimulated gal3⁺/⁺ and gal3⁻/⁻ BMMC. C, gal3⁺/⁺ and gal3⁻/⁻ BMMC sensitized with anti-DNP IgE and then activated with DNP-BSA for 12 and 24 h. D, Three-week-old gal3⁺/⁺ and gal3⁻/⁻ BMMC incubated with SCF for 2 wk. E, Cells obtained from lavage of the peritoneal cavity of gal3⁺/⁺ and gal3⁻/⁻ mice were cultured in the presence of SCF for 3 wk. F, gal3⁺/⁺ and gal3⁻/⁻ embryonic fibroblasts.
originating from the peritoneal cavity and expanded in the presence of SCF. As shown in Fig. 4E, the association between low JNK1 expression and galectin-3 deficiency is retained. To determine whether the defective JNK1 expression in gal3−/− mice is cell type specific, we tested the JNK1 levels in other cell types from gal3+/+ and gal3−/− mice. We did not see any difference in JNK1 levels in embryonic fibroblasts (Fig. 4F), as well as in resting and activated macrophages and lymphocytes (data not shown).

gal3−/− BMMC are defective in activation of c-Jun

Consistent with the defective JNK1 expression, phosphorylation of c-Jun was significantly lower in gal3−/− BMMC compared with gal3+/+ BMMC that were activated by FcεRI cross-linkage (Fig. 5A). In addition, we precipitated JNK1 in the cell lysates and quantitated the c-Jun kinase activity in the precipitates by an in vitro kinase assay. As shown in Fig. 5B, immunoprecipitates from gal3−/− BMMC had significantly reduced ability to phosphorylate c-Jun compared with gal3+/+ BMMC. Comparison of the relative amounts of phosphorylated c-Jun with those of JNK1 between the two genotypes showed that JNK1 in gal3−/− BMMC had lower kinase activity compared with that in gal3+/+ BMMC.

gal3−/− BMMC have reduced IL-4 mRNA and JNK mRNA

Because IL-4 production is controlled by JNK at the transcription level, our results suggested that the decreased IL-4 production in gal3−/− BMMC may be due to a reduced level of transcription of this cytokine. By using real-time PCR, we compared the IL-4 mRNA levels in gal3+/+ and gal3−/− BMMC that were activated by FcεRI cross-linkage. As shown in Fig. 6A, the level was significantly lower in gal3−/− BMMC than gal3+/+ BMMC.

As a first step in elucidating the mechanism by which JNK expression is regulated by galectin-3, we determined whether transcriptional regulation is involved. We compared the mRNA levels of JNK1 in gal3+/+ and gal3−/− BMMC by RT-PCR. The results showed that the basal level of JNK1 mRNA was lower in gal3−/− BMMC compared with gal3+/+ BMMC (Fig. 6B). There was a rapid induction of JNK1 mRNA in both genotypes upon activation by FcεRI cross-linkage. However, the level in gal3−/− BMMC remained considerably lower than that in gal3+/+ BMMC (Fig. 6B).

FIGURE 5. gal3−/− BMMC exhibit lower c-Jun activation. A, BMMC were sensitized with anti-DNP IgE and then activated with DNP-BSA for the indicated periods. The levels of phosphorylation of c-Jun were determined by immunoblotting using anti-phospho-c-Jun. B, BMMC were sensitized with anti-DNP IgE and then activated with DNP-BSA for the indicated periods. The JNK kinase activity in the lysates was analyzed by using GST-c-Jun as substrate. The activities relative to the amount of JNK1 are listed underneath the bands.

FIGURE 6. gal3−/− BMMC exhibit lower levels of mRNA for IL-4 and JNK1. A, BMMC were activated by incubating in microtiter wells coated with anti-DNP IgE or PBS (control) and the levels of IL-4 mRNA were determined by real-time PCR. B, RT-PCR analysis of JNK mRNA levels in BMMC sensitized with anti-DNP IgE and then activated with DNP-BSA. After various periods, the JNK mRNA levels were analyzed by RT-PCR.

To determine whether galectin-3 controls JNK1 gene transcription, we tested the effect of galectin-3 on the activation of JNK promoter by a reporter assay. We did not detect induction of JNK promoter by galectin-3 in gal3−/− BMMC cotransfected with JNK promoter-reporter construct and galectin-3 (data not shown).

Suppression of IL-4 production in BMMC by a JNK inhibitor

Although JNK is known to mediate cytokine production in T cells, its role in IL-4 production in mast cells has not been demonstrated. To confirm that defective JNK1 synthesis and activation as seen in gal3−/− mast cells can result in reduced IL-4 production, we tested whether inhibition of JNK1 can suppress IL-4 production. gal3+/+ and gal3−/− BMMC were activated in the presence of a serially diluted JNK inhibitor, SP600125 (35). As shown in Fig. 7, IL-4 release in both gal3+/+ and gal3−/− BMMC was inhibited by this drug in a dose-dependent fashion.

gal3+/+ and gal3−/− BMMC do not differ significantly in signaling pathways linked to secretory granule exocytosis

Because JNK1 is not known to be involved in the signaling events associated with mast cell granule exocytosis, we conducted experiments to determine how galectin-3 contributes to this process. Galectin-3 does not appear to regulate some upstream events, such as activation of receptor-associated tyrosine kinases, because tyrosine phosphorylation of both Lyn and Syk were comparable in
gal3+/+ and gal3−/− BMMC activated by FcεRI cross-linkage (data not shown). We then examined calcium mobilization induced by FcεRI cross-linkage and did not notice a difference between gal3+/+ and gal3−/− BMMC (Fig. 8A). We next studied phosphorylation of PLCγ1 and an adaptor molecule, LAT, and did not find significant differences (Fig. 8B). It is unlikely that galectin-3 is involved in activation of another adaptor molecule, Src homology protein of 76 kDa (SLP-76), because this protein as well as LAT has an essential role in calcium mobilization and galectin-3 appears not to be involved in this latter process. Finally, we examined the activation of PKCβ, which is implicated in degranulation and noted that a similar extent of phosphorylation of PKCβ was achieved in gal3+/+ and gal3−/− BMMC upon FcεRI cross-linkage (Fig. 8C).

Discussion

The functions of galectin-3 have previously been studied mainly by treating various cells with exogenously added recombinant protein, and by this approach, galectin-3 has been shown to be able to activate various cell types, including mast cells (17). The availability of genetically engineered galectin-3-deficient mice has made it possible to study the role of endogenous galectin-3 in various cell types. Our results show the involvement of galectin-3 in the FcεRI-mediated mast cell response. BMMC from gal3−/− mice exhibited significantly reduced degranulation (i.e., histamine release) and lower cytokine (IL-4) production upon FcεRI cross-linkage. Importantly, gal3−/− mice exhibit a significantly diminished cutaneous mast cell response in vivo, as demonstrated by PCA reactions.

Like all other members of the galectin family, galectin-3 does not contain a classical signal sequence. Nevertheless, it can be secreted from various cell types (5), including mouse macrophage cell lines stimulated with a calcium ionophore (36). However, the possibility that galectin-3 released by mast cells in turn activates neighboring cells in a paracrine fashion, through binding to cell surface glycoconjugates, is excluded by the following experimental results. We activated gal3−/− BMMC by FcεRI cross-linkage in the presence of lactose, which would bind to extracellular galectin-3 and inhibit its activity, or with another disaccharide, sucrose, which does not bind to the lectin. We found that IL-4 production was not affected by the presence of either sugar (data not shown). These results strongly suggest the involvement of galectin-3 in mast cell signaling through an intracellular mechanism.

Based on our results, some potential causes of lower degranulation and cytokine production in gal3−/− BMMC can be excluded. First, the deficiency is not due to a reduction in the number of FcεRI on the cells. Second, it is not due to lack of differentiation of these cells, as we found comparable levels of cell surface c-Kit (Fig. 1C) and mRNA of a mast cell-specific protease, MCP-5 (data not shown) in cells from the two genotypes. Third, galectin-3 does not appear to regulate some upstream events, such as activation of receptor-associated tyrosine kinases, because tyrosine phosphorylation of both Lyn and Syk were comparable in gal3+/+ and gal3−/− BMMC activated by FcεRI cross-linkage (data not shown).

Our present study provides a significant mechanistic insight into the regulation of mast cell cytokine production by galectin-3 and that is the involvement of this lectin in regulation of JNK1 expression. JNK1 is known to play an important role in the production of cytokines from T cells by activating the transcription factor c-Jun, which is in turn involved in transcription of various cytokines (37). Thus, the reduced cytokine production in gal3−/− BMMC is likely due to the lower JNK1 level. It is to be noted that only one of the two isoforms of JNK1 is affected, and this could explain a partial reduction in IL-4 production as the other isoform is likely to regulate the production of this cytokine also.

The basis for galectin-3’s regulation of JNK1 expression remains to be elucidated. Our finding that gal3−/− BMMC contains significantly lower JNK1 transcript suggests that galectin-3 regulates JNK1 transcription. Galectin-3 has been shown to interact with a thyroid transcription factor, TTF, and potentiate the activity of this factor (38). It is conceivable that galectin-3 regulates the transcription factor involved in the expression of JNK1. However, our experiments with a reporter assay did not support the control of JNK promoter activity by galectin-3 (data not shown). The finding that only one isoform is affected is unusual. Because these isoforms are products of alternative splicing, the intriguing possibility exists that galectin-3 regulates differential splicing of JNK pre-mRNA. In this regard, it is interesting to note that galectin-3, as well as galectin-1, has been shown to be active in inducing pre-mRNA splicing in vitro (39).

How galectin-3 regulates degranulation needs to be considered, since this process is not known to be dependent on JNK1. We studied a number of signaling events and molecules known to contribute to secretory granule exocytosis, including calcium influx, phosphorylation of PLCγ1, and the adaptor molecule LAT, and activation of PKCβ and did not notice significant alterations in gal3−/− BMMC. We cannot exclude the possibility that galectin-3...
regulates activation of the soluble N-ethylmaleimide-sensitive factor attachment receptor complex that is involved in the membrane fusion during degranulation (40). Galectin-3 has recently been shown to be present in the lumen of intracellular vesicles containing glycoproteins destined to the apical site of epithelial cells (41). A model has been proposed in which galectin-3 is responsible for clustering these apical glycoproteins and facilitating the generation of apical vesicles. Because galectin-3 is present in secretory granules in mast cells (16), an intriguing possibility exists that galectin-3 is involved in clustering of glycoproteins contained in the granules and thus facilitating the targeting of these granules to the plasma membrane before exocytosis.

Our results strengthen the notion that the galectins can function intracellularly. Such functions are unexpected from the lectin properties, but are consistent with their intracellular localization (42). These functions likely involve interactions with intracellular proteins, which could be dependent or independent of carbohydrates. Saccharides, especially the O-linked varieties, do exist in the cytoplasm and may potentially be ligands of cytosolic galectins. However, there is evidence that galectin-3 interacts with proteins that are not glycoproteins, suggesting the involvement of carbohydrate-independent protein-protein interactions (reviewed in Ref. 8).

In conclusion, our studies have established an important role for galectin-3 in the mast cell response. In view of the well-established significance of mast cells in allergic inflammation, our studies suggest that galectin-3 could be a potential therapeutic target for controlling allergic disorders.

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Disclosures
The authors have no financial conflict of interest.

References
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