



Down-modulation of antigen-induced activation of murine cultured mast cells sensitized with a highly cytokinerigic IgE clone



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ARTICLE INFO

Article history:

Received 9 October 2015

Received in revised form 5 April 2016

Accepted 5 April 2016

Available online 6 April 2016

Keywords:

Mast cell

IgE

JNK

Degranulation

Allergy

ABSTRACT

Accumulating evidence suggests that several IgE clones can activate mast cells during the sensitization phase even in the absence of antigen. They were found to induce pro-inflammatory cytokine release, histamine synthesis, chemotaxis, adhesion, and accelerated maturation of mast cells, although it remains unknown whether antigen-induced responses can be affected by differences of IgE clones. We compared two IgE clones, which were different in the capacity to activate mast cells during sensitization, in terms of potentials to affect antigen-induced degranulation and cytokine releases using IL-3-dependent murine bone marrow-derived cultured mast cells (BMMCs). Antigen-induced degranulation and pro-inflammatory cytokine release were augmented, when BMMCs were sensitized with elevated concentrations of a clone IgE-3, which did not induce phosphorylation of JNK and cytokine release in the absence of antigen, whereas those were significantly rather decreased, when BMMCs were sensitized with elevated concentrations of a clone SPE-7, one of the most potent cytokinerigic IgE clones, which intensively induced phosphorylation of JNK. This attenuated response with SPE-7 was accompanied by decreased tyrosine phosphorylation of the cellular proteins including Syk upon antigen stimulation. SP600125, which is known to inhibit JNK, restored the levels of antigen-induced degranulation and phosphorylation of Syk in BMMCs sensitized with higher concentrations of a clone SPE-7 when it was added before sensitization. Treatment with anisomycin, a potent activator of JNK, before IgE sensitization significantly suppressed antigen-induced degranulation. These findings suggest that differences of sensitizing IgE clones can affect antigen-induced responses and activation of JNK during sensitization might suppress antigen-induced activation of mast cells.

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1. Introduction

Mast cells play critical roles in immediate allergy, in which they are activated by IgE-mediated antigen stimulation. Activated mast cells release a wide variety of mediators, such as histamine, granule proteases, arachidonic acid metabolites, and pro-inflammatory cytokines, which modulate the inflammatory responses at multiple stages [1]. Since elevated production of IgE has often been observed in subjects with chronic allergic inflammation including the atopic dermatitis, particular attention has been paid to the

relationship between IgE and mast cells. Accumulating evidence suggests that IgE molecules have potentials to influence mast cell functions even in the absence of the cognate antigen and that the potency is much varied among different IgE clones. Early studies demonstrated that IgE can up-regulate the surface expression levels of FcεRI in basophils and mast cells [2,3]. Drastic decreases in the surface expression of FcεRI were found in the peritoneal mast cells of the IgE^{-/-} mice [3]. IgE-elicited up-regulation of the surface FcεRI was found to be mediated largely through its increased stabilization at the plasma membranes [4,5]. Antigen-independent effects of IgE on mast cells were also demonstrated in cultured mast cells lacking the Src homology 2-containing inositol phosphatase (SHIP): IgE induced a massive Ca²⁺ influx and degranulation in the absence of antigens, raising the possibility that IgE binding might activate

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mast cells [6]. Two groups subsequently demonstrated using IL-3-dependent bone marrow-derived cultured mast cells (BMMCs) that IgE has a protective role from apoptotic cell death induced by IL-3 withdrawal [7,8]. The following studies including ours revealed that IgE could evoke a wide spectrum of responses in mast cells including cytokine production, histamine synthesis, adhesion, and chemotaxis, in the absence of antigens [8–12]. These studies using various murine IgE clones demonstrated that not all IgE clones can activate mast cell in the absence of antigens. Although it remains to be clarified why several specific clones can efficiently activate mast cells in the absence of antigens, it is generally accepted that antigen-independent effects of IgE (monomeric IgE) should be involved at least in part in exacerbation of chronic inflammatory responses [13]. Recently, Bax et al. demonstrated using the Fab fragment that cross-linking of FcεRI by free IgE molecules should be involved in the antigen-independent effects of an IgE clone, SPE-7 [14].

Elevated serum IgE levels are one of common indicators of immune dysregulation [15,16]: chronic allergic diseases, such as allergic asthma and atopic dermatitis, are often accompanied by increased serum IgE levels. In addition to systemic IgE elevation, local IgE synthesis in the nasal mucosa in allergic rhinitis was also reported [17]. Accumulating evidence indicates that mast cells undergo transient activation during the sensitization phase while it remains largely unknown how these IgE clones affect the characteristics of mast cells, such as differentiation and degranulation in response to secretagogues. Bryce et al. demonstrated that impaired hapten sensitization in IgE-deficient mice was restored by exogenous IgE molecules in the oxazolone-induced contact hypersensitivity model even when the added IgE clone did not recognize the contact allergen, implying the antigen-independent immune modulatory roles of IgE [18]. Recently, highly cytokinergic IgE clones were found to accelerate growth and differentiation of murine bone marrow-derived mast cell progenitors *in vitro*, raising the possibility that elevated IgE levels can affect the number and phenotypic feature of tissue mast cells [19].

Our purpose in this study is to determine the monomeric IgE effects on antigen-induced activation of mast cells. In contrast with several studies that indicated the positive effects of elevated concentrations of IgE on antigen-induced activation of mast cells, we demonstrate here that the maximal responses induced upon antigen stimulation are significantly decreased when mast cells are sensitized with higher concentrations of highly cytokinergic IgE, suggesting that the monomeric IgE effects on antigen-induced activation of mast cells are more complicated than expected.

2. Materials and methods

2.1. Mice

Specific-pathogen-free, 8–12 week-old male Balb/c mice were obtained from Japan SLC (Hamamatsu, Japan), and all mice were kept in a specific-pathogen-free animal facility at Kyoto University, Mukogawa Women's University and Okayama University. All experiments conformed to the guidelines on the ethical use of animals set by the U. S. National Institutes of Health and were approved by the Committee on Animal Experiments of Kyoto University, Mukogawa Women's University and Okayama University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Materials

The following materials were commercially obtained from the sources indicated: *p*-nitrophenyl-β-D-2-acetoamide-2-deoxyglucopyranoside, an anti-dinitrophenyl IgE antibody

(clone SPE-7), dinitrophenyl human serum albumin (DNP-HSA, DNP/albumin (molar ratio)=30–40), and anisomycin from Sigma-Aldrich (St. Louis, MO), trinitrophenyl bovine serum albumin (TNP-BSA, TNP/albumin (molar ratio)=25 ± 10) from LSL (Tokyo, Japan), SP600125 from Biomol (Plymouth Meeting, PA), LY294002 from Calbiochem (La Jolla, CA), recombinant mouse IL-3 from R&D Systems (Minneapolis, MN), an anti-actin antibody from Merck Millipore (Billerica, MA), an anti-trinitrophenyl IgE antibody (clone IgE-3), an anti-JNK1/2 antibody, and an FITC conjugated anti-IgE antibody from BD Pharmingen (San Diego, CA), an anti-phosphorylated JNK antibody from Promega (Madison, WI), an anti-phosphorylated ERK (p42/44) antibody, an anti-ERK (p42/44) antibody, an anti-phosphorylated p38 MAPK antibody, an p38 MAPK antibody, an anti-phosphorylated Syk (Tyr525/526) antibody, and an anti-Syk antibody from Cell Signaling Technology (Beverly, MA), and an anti-phosphotyrosine antibody (clone 4G10) from Upstate Technology (Lake Placid, NY). All other chemicals were commercial products of reagent grade. We confirmed reproducibility of the results using the different batch of each IgE clone.

2.3. Preparation of BMMCs

Preparation of BMMCs was performed as described [20]. Briefly, bone marrow cells obtained from Balb/c mice were cultured in the presence of 10 ng/ml IL-3 for ~30 days. Greater than 95% of the cells were confirmed as mast cells by the acidic Toluidine blue staining.

2.4. Measurement of degranulation

BMMCs were sensitized with each IgE clone for 24 h, washed three times, and then stimulated with its cognate antigen for 30 min in 25 mM PIPES-NaOH, pH 7.4 containing 125 mM NaCl, 2.7 mM KCl, 5.6 mM glucose, 1 mM CaCl₂, and 0.1% bovine serum albumin. Degranulation of mast cells was evaluated by measuring enzyme activity of a lysosomal enzyme, β-hexosaminidase, using a substrate, *p*-nitrophenyl-β-D-2-acetoamide-2-deoxyglucopyranoside. We used more than three batches of each IgE clone and confirmed comparable results in antigen-induced responses.

2.5. Measurement of cytokine release

Inflammatory cytokines (IL-6, TNF-α, and CCL2) were measured by ELISA kits (OptEIA, BD Biosciences, San Diego, CA) according to the manufacturer's instructions, respectively.

2.6. Flow cytometry

The cells were pretreated with an anti-mouse CD16/32 antibody (clone 2.4G2) to block IgG binding to Fcγ receptors for 10 min and then sensitized with the clone SPE-7 at 4 °C for 50 min. The cells were washed and labeled using FITC-conjugated anti-mouse IgE. Flow cytometry was performed as previously described with FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) equipped with CELLQUEST software [21].

2.7. Immunoblot analysis

Immunoblot analysis was performed as described previously [22].

2.8. Statistical analysis

Data are presented as the means ± SEM. Statistical significance for comparisons between groups was determined using one-way

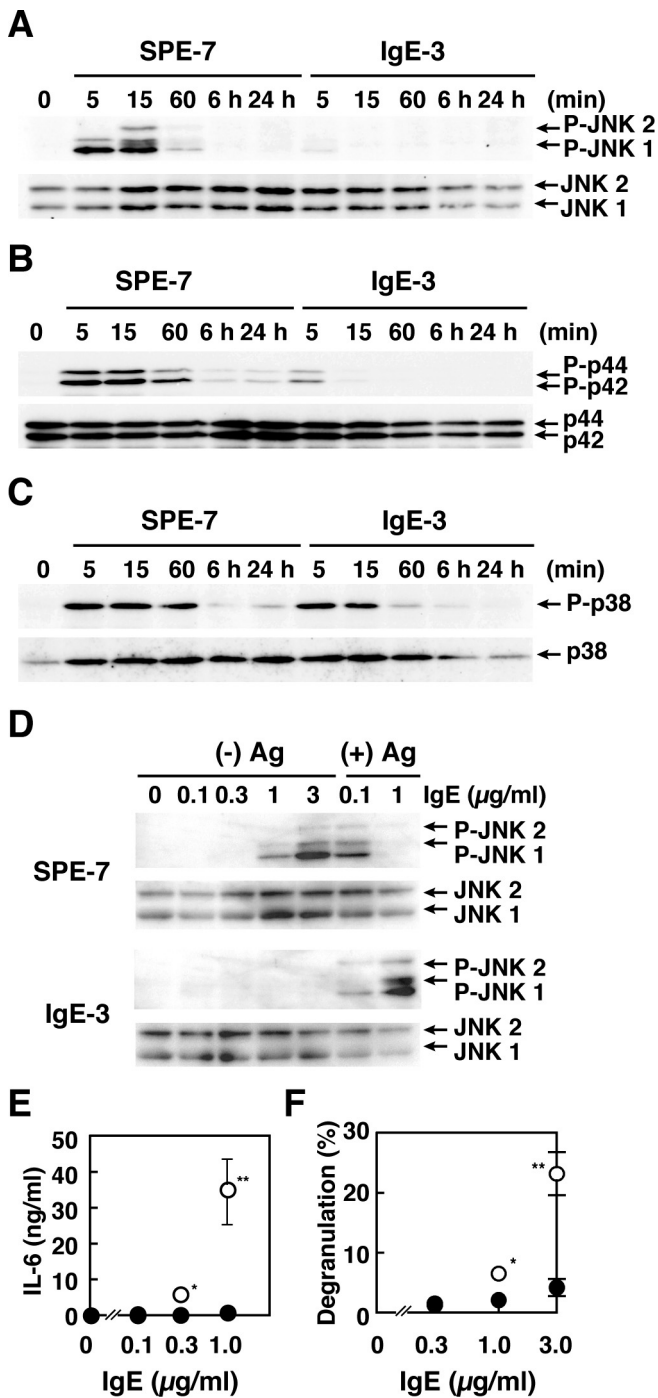


Fig. 1. Activation of BMMCs stimulated with IgE alone. (A–C) BMMCs were stimulated with 1.0 μg/ml IgE (clone SPE-7 and IgE-3) for the indicated periods. Phosphorylation of JNK (A), ERK (B), and p38 MAPK (C) was investigated using the specific antibodies. (D) Phosphorylation levels of JNK were compared between the cells stimulated with IgE and those with the antigen. (E) BMMCs were stimulated with the indicated concentrations of IgE (open circles, SPE-7, and closed circles, IgE-3) for 3 h and the amounts of IL-6 in the medium were measured. (F) BMMCs were stimulated with the indicated concentrations of IgE (open circles, SPE-7, and closed circles, IgE-3) for 30 min and the levels of degranulation were determined by measuring β-hexosaminidase activity. Values are presented as the means ± SEMs (n = 3). Values with **p* < 0.05 and ***p* < 0.01 (vs. IgE-3) are regarded as significant.

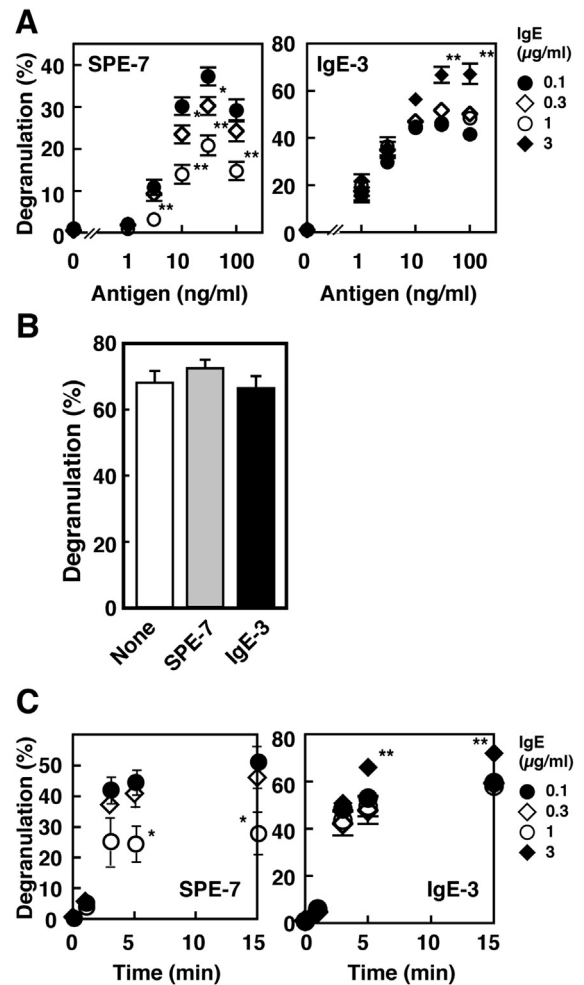


Fig. 2. Suppression of antigen-induced degranulation in BMMCs sensitized with higher concentrations of SPE-7. (A) BMMCs were sensitized with various concentrations (closed circles, 0.1 μg/ml, open diamonds, 0.3 μg/ml, open circles, 1.0 μg/ml, closed diamonds, 3 μg/ml) of the two different IgE clones, SPE-7 and IgE-3, for 24 h respectively, and then stimulated with the indicated concentrations of the specific antigen (DNP-HSA and TNP-BSA) for 30 min. The levels of degranulation were determined by measuring β-hexosaminidase activity. Values are presented as the means ± SEMs (n = 10). Values with **p* < 0.05 and ***p* < 0.01 (vs. 0.1 μg/ml IgE) are regarded as significant. (B) BMMCs sensitized with 1 μg/ml IgE for 24 h were stimulated with 300 nM thapsigargin. Values are presented as the means ± SEMs (n = 3). (C) The time course of β-hexosaminidase release was investigated. BMMCs were sensitized with each IgE clone as described above and stimulated its specific antigen (30 ng/ml). Values are presented as the means ± SEMs (n = 3). Values with **p* < 0.05 (vs. 0.1 μg/ml IgE) are regarded as significant.

ANOVA. Additional comparisons were made with Dunnett multiple comparison test for comparison with the control groups or Tukey-Kramer multiple comparison test for all pairs of column comparison.

3. Results

3.1. Comparison of two different murine IgE clones in mast cell activation

We first made comparisons of the potentials for mast cell activation between two different IgE clones, SPE-7 and IgE-3. SPE-7 is one of the best-characterized murine IgE clones and has been regarded as a typical highly cytokinergic IgE clone [23]. SPE-7 induced significant phosphorylation of JNK and ERK in BMMCs whereas IgE-3 did only marginal responses (Fig. 1A and B). Phosphorylation levels of p38 MAPK were decreased in the cells treated with IgE-3 at the

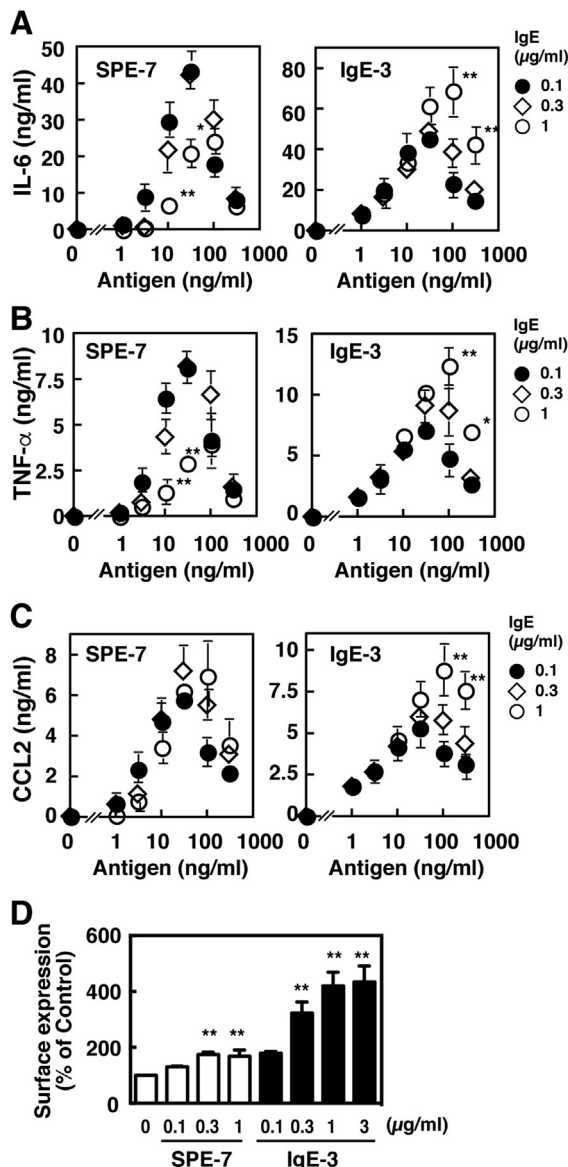


Fig. 3. Suppression of inflammatory cytokine production in BMMCs sensitized with SPE-7. (A–C) BMMCs were respectively sensitized with various concentrations (closed circles, 0.1 μg/ml, open diamonds, 0.3 μg/ml, and open circles, 1.0 μg/ml) of the two different IgE clones, SPE-7 and IgE-3, for 24 h, and then stimulated with the indicated concentrations of the specific antigen (DNP-HSA and TNP-BSA) for 3 h. The amount of various cytokine release was investigated (A, IL-6, B, TNF-α, and C, CCL2). Values are presented as the means ± SEMs (n = 4). Values with **p* < 0.05 and ***p* < 0.01 (vs. 0.1 μg/ml IgE) are regarded as significant. (D) BMMCs were sensitized with the indicated concentrations of IgE (open columns; SPE-7, closed columns; IgE-3) for 24 h. The surface expression of FcεRI was measured by flow cytometry with an FITC-conjugated anti-IgE antibody. The relative expression levels are presented as the means ± SEMs (n = 3, the expression level without sensitization = 100). Values with **p* < 0.05 and ***p* < 0.01 (vs. 0 μg/ml IgE) are regarded as significant.

time points, 60 min and 24 h (Fig. 1C). Phosphorylation levels of JNK induced by SPE-7 were comparable to those upon antigen stimulation whereas those by IgE-3 were quite lower than those upon antigen stimulation (Fig. 1D). SPE-7 induced massive IL-6 production and detectable degranulation at higher concentrations (Fig. 1E and F), whereas IgE-3 failed to induce IL-6 production and only induced marginal levels of degranulation at higher concentrations. These results were consistent with the previous studies; SPE-7 is a highly cytokinergic clone whereas IgE-3 is a poorly cytokinergic one [11,19].

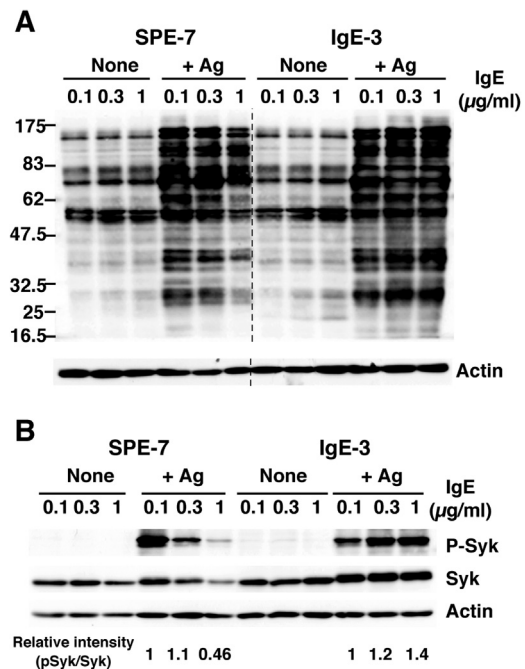


Fig. 4. Decreased protein tyrosine phosphorylation upon antigen stimulation in BMMCs sensitized with increasing concentrations of SPE-7. BMMCs were sensitized with 0.1, 0.3, and 1.0 μg/ml of the two different IgE clones, SPE-7 and IgE-3, for 24 h respectively, and then stimulated with (+Ag) or without (None) the specific antigen (30 ng/ml, DNP-HSA and TNP-BSA) for 5 min (C) or 15 min (A and B). The levels of protein tyrosine phosphorylation were investigated using an anti-phosphotyrosine antibody (A, clone 4G10), and an anti-phosphorylated Syk antibody (B). The amount of actin was measured as the loading control.

3.2. Suppression of antigen-induced degranulation and pro-inflammatory cytokine production in mast cells sensitized with higher concentrations of SPE-7

We then investigated the levels of antigen-induced degranulation of BMMCs sensitized with various concentrations of IgE. The profiles of antigen-induced degranulation were very similar among the cells sensitized with various concentrations of IgE-3, except that the maximal levels were significantly higher in the cells sensitized with 3 μg/ml IgE-3 (Fig. 2A). In contrast, the maximal levels of degranulation were significantly decreased in the cells sensitized with higher concentrations of SPE-7. The levels of degranulation induced by thapsigargin were comparable among the cells sensitized with or without 1 μg/ml each IgE clone, indicating that the capacity of degranulation were not changed in the cells sensitized with 1 μg/ml SPE-7 (Fig. 2B). Time course studies also indicated that the maximal responses declined in the cells sensitized with 1 μg/ml SPE-7 (Fig. 2C).

We then measured antigen-induced production of pro-inflammatory cytokines, such as IL-6, TNF-α, and CCL2. Production of these cytokines was comparable among the cells sensitized with various concentrations of IgE-3, except that the maximal levels were significantly higher in the cells sensitized with 1 μg/ml IgE-3 (Fig. 3A–C). In contrast, the maximal production of IL-6 and TNF-α, not of CCL2, was significantly decreased in the cells sensitized with 1 μg/ml SPE-7 in comparison with those with 0.1 μg/ml SPE-7. Surface expression of FcεRI, was drastically up-regulated in the cells sensitized with higher concentrations of IgE-3, whereas that was moderately but significantly increased in the cells sensitized with SPE-7 (Fig. 3D).

3.3. Decreased tyrosine phosphorylation upon antigen stimulation in mast cells sensitized with higher concentrations of SPE-7

Antigen-induced tyrosine phosphorylation of the cellular proteins was attenuated in the cells sensitized with higher concentrations of SPE-7 whereas that was rather augmented in those sensitized with higher concentrations of IgE-3 (Fig. 4A). The overall suppression of tyrosine phosphorylation suggested possible defects in the upstream of the FcεRI-mediated signal transduction pathway. Indeed, antigen-induced phosphorylation of Syk was drastically suppressed in the cells sensitized with 1 μg/ml SPE-7 in comparison with those with 0.1 μg/ml SPE-7 (Fig. 4B). Similar changes in antigen-induced phosphorylation of JNK were also observed (Fig. 1D).

3.4. Activation of JNK during the sensitization phase is involved in suppression of antigen-induced degranulation of mast cells

These results suggested that SPE-7-mediated changes during the sensitization phase should result in suppression of several responses upon antigen stimulation. We found that SP600125, which is often used as a JNK inhibitor, could restore antigen-induced degranulation in the cells sensitized with higher concentrations of SPE-7. When the cells were treated with SP600125 before sensitization with SPE-7, the levels of antigen-induced degranulation were significantly restored (Fig. 5A). Since a previous study demonstrated that SP600125 could inhibit phosphatidylinositol-3 kinase (PI3K) activity at high concentrations in mast cells [24], it is possible that the effects of SP600125 is mediated by inhibition of PI3K. However, a specific inhibitor of PI3K, LY294002, did not mimic the SP600125-mediated restoration of degranulation responses (Fig. 5A). Treatment with SP600125 before sensitization also restored tyrosine phosphorylation of Syk upon antigen stimulation in the cells sensitized with 1 μg/ml SPE-7, whereas antigen-induced phosphorylation of Syk remains unchanged in the cells sensitized with IgE-3 with or without treatment with SP600125 (Fig. 5B). On the other hand, when the cells were treated with SP600125 right before antigen stimulation, antigen-induced degranulation was significantly suppressed, indicating that treatment with SP600125 results in opposite effects on antigen-induced degranulation depending on the timing of addition (Fig. 5A and C).

Phosphorylation of ERK was also detected in the cells sensitized with SPE-7, not with IgE-3 (Fig. 1B), raising the possibility that ERK is also involved in SPE-7-mediated changes in antigen-induced activation. U0126, which is often used as a MEK inhibitor, could not restore antigen-induced degranulation in the cells sensitized with higher concentrations of SPE-7 (Fig. 6A). On the other hand, when the cells were treated with U0126 right before antigen stimulation, antigen-induced degranulation was significantly suppressed (Fig. 6B).

3.5. Anisomycin treatment before sensitization mimicked the effects of SPE-7

We then investigated the effects of one of the typical JNK activators, anisomycin, on antigen-induced degranulation. In BMMCs, anisomycin-induced phosphorylation of JNK were augmented in a dose-dependent manner, the maximum of which was comparable to the level in the cells stimulated with 1 μg/ml SPE-7 (Fig. 7A). When the cells were pretreated with anisomycin for 10 min before sensitization with IgE-3, antigen-induced degranulation was significantly decreased (Fig. 7B), raising the possibility that activation of JNK during the sensitization phase might suppress the following antigen-induced degranulation. Because anisomycin is known

to inhibit protein synthesis [25], we investigated the effects of cycloheximide on degranulation. Pretreatment of BMMCs with cycloheximide (1, 3, and 10 μg/ml, 30 min) before sensitization with IgE-3 had no effects on antigen-induced degranulation (data not shown), excluding the possibility that inhibition of protein synthesis is involved in the anisomycin-mediated inhibition.

4. Discussion

Accumulating evidence suggests that several IgE clones should potentiate the pro-inflammatory function of immature mast cells, such as pro-inflammatory cytokine production, histamine synthesis, chemotaxis, survival, and accelerated maturation even in the absence of antigens [2,7–12,23]. IgE was found to increase surface expression levels of FcεRI and thereby enhance the antigen-induced responses in mast cells. Yamaguchi et al. reported that 4 days of treatment of BMMCs with IgE drastically up-regulated surface expression of FcεRI and significantly augmented the levels of degranulation and release of IL-4 and IL-6 [3]. In human umbilical cord blood-derived cultured mast cells, similar prolonged sensitization with human myeloma IgE resulted in increased antigen-induced degranulation [26]. These findings suggest that sensitization of mast cells with higher concentrations (5 μg/ml) of IgE should enhance the antigen-induced responses through up-regulation of surface expression of FcεRI. In this study, the clone, IgE-3, drastically up-regulated the surface expression of FcεRI and augmented the levels of degranulation and cytokine release induced by the antigen, which was largely consistent with these previous reports. In contrast, high concentrations of the IgE clone, SPE-7, rather down-modulated the degranulation and release of IL-6 and TNF-α, in comparison with the low concentrations. This result suggests that highly cytokinergic IgE, such as the clone, SPE-7, can induce release of a variety of pro-inflammatory cytokines on the onset of sensitization, but dampen the antigen-induced responses. It might raise the possibility that antigen-induced activation of mast cells is affected by the nature of bound IgE as well as monomeric IgE responses.

Our findings indicated that decreased responses in SPE-7-sensitized BMMCs upon antigen stimulation should result from attenuated tyrosine phosphorylation downstream of FcεRI, including phosphorylation of Syk and JNK. Because Syk plays critical roles in antigen-induced activation of mast cells [27], decreased phosphorylation of Syk might result in impaired degranulation and cytokine release. We found that SP600125 could restore the phosphorylation levels of Syk in antigen-stimulated BMMCs sensitized with higher concentration of SPE-7. SP600125 could also ameliorate the impaired degranulation, raising the possibility that JNK, which is highly phosphorylated upon sensitization with SPE-7, should play critical roles in suppression of antigen-induced degranulation. Bain et al. demonstrated using in vitro kinase assay systems that SP600125 can inhibit a broad spectrum of the other protein kinases, such as p70 ribosomal protein S6 kinase, serum/glucocorticoid regulated kinase 1, AMP-activated protein kinase, and cyclin-dependent kinase 2, casein kinase 1, Aurora B, Aurora C, and checkpoint kinase 2 [28,29], raising the possibility that the action of SP600125 is mediated by these other kinases. However, we propose that JNK should be involved in suppression of antigen-induced activation of BMMCs sensitized with highly cytokinergic IgE clones, because anisomycin treatment before IgE sensitization, which induced phosphorylation of JNK, resulted in significant suppression of antigen-induced degranulation of BMMCs sensitized with IgE-3. JNK1 was found to phosphorylate p66Shc at Ser36 in response to ultraviolet irradiation [30] and this phosphorylation might be essential for p66Shc-mediated suppression of antigen-induced activation of mast cells [31]. Ulivieri

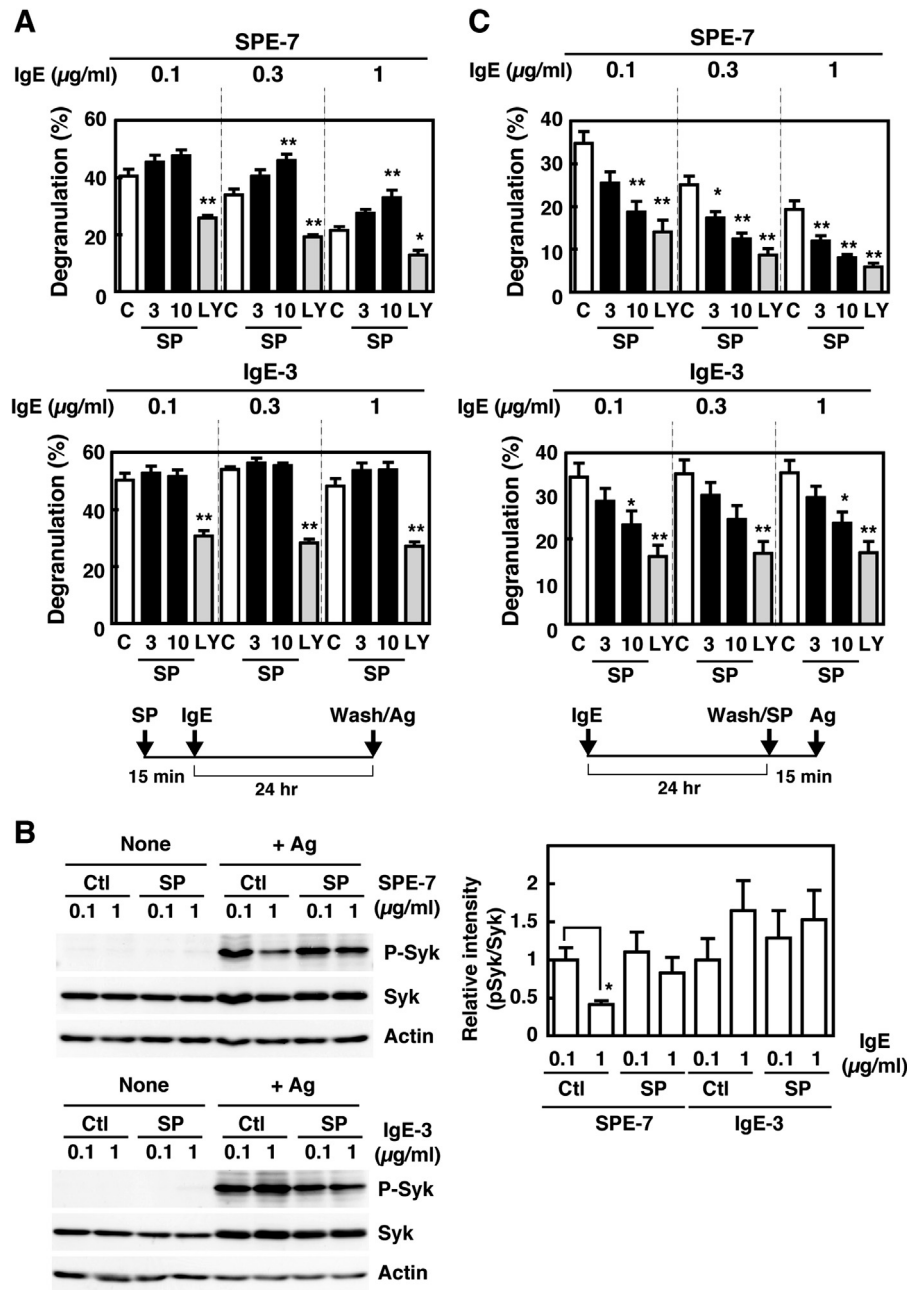


Fig. 5. Restoration of antigen-induced degranulation by SP600125 during the sensitization phase. (A) BMMCs pre-incubated with or without the indicated concentrations of SP600125 (SP, µM) for 15 min were sensitized with the indicated concentrations of IgE (SPE-7 and IgE-3) for 24 h, and then stimulated with 30 ng/ml of the specific antigen. Values are presented as the means ± SEMs (n = 6). Values with ***p* < 0.01 (vs. Control, C) are regarded as significant. (B) BMMCs pre-incubated without (Ctl) or with 10 µM SP600125 (SP) for 15 min were sensitized with the indicated concentrations of IgE (upper panels, SPE-7 and lower panels, IgE-3) for 24 h, and then stimulated without (None) or with 30 ng/ml of the specific antigen (+Ag). Tyrosine phosphorylation of Syk was measured as described in the legend to Fig. 4. The relative amounts of the phosphorylated Syk (pSyk/Syk) were calculated based on the densitometry. Values are presented as the means ± SEMs (n = 4). A value with **p* < 0.05 is regarded as significant. (C) BMMCs were sensitized with the indicated concentrations of IgE (SPE-7 and IgE-3) for 24 h, and washed to remove unbound IgE. The cells were incubated with the indicated concentrations of SP600125 (SP) for 15 min, and then stimulated with 30 ng/ml of the specific antigen. Values are presented as the means ± SEMs (n = 5). Values with **p* < 0.05 and ***p* < 0.01 (vs. Control, C) are regarded as significant. The levels of degranulation were determined by measuring β-hexosaminidase activity (A and C). A specific inhibitor of PI3 K, LY294002 (50 µM, LY) was used for comparison (A and C).

et al. demonstrated that p66Shc could form a complex with SHIP1 and inhibit the signal transduction upon antigen stimulation. They demonstrated using exogenous gene expression analyses with a rat mast cell line, RBL-2H3, that phosphorylation of p66Shc at Ser36 was required for SHIP1-mediated suppression [31]. It is likely that JNK-mediated phosphorylation at Ser36 enhances the potentials of p66Shc to suppress antigen-induced activation of BMMCs. On the other hand, lipopolysaccharide-mediated production of IL-10 in BMMCs was found to be suppressed by curcumin, which can act as a

JNK inhibitor, and dominant negative forms of JNK [32], raising the possibility that activation of JNK leads to production of IL-10, which can suppress mast cell activation [33]. We investigated this possibility but failed to detect IL-10 production in BMMCs sensitized with SPE-7 (data not shown).

In contrast, when SP600125 was added right before antigen stimulation, it significantly suppressed degranulation of mast cells, raising the possibility that JNK plays positive roles in degranulation. Since Tanemura et al. reported that SP600125 can significantly

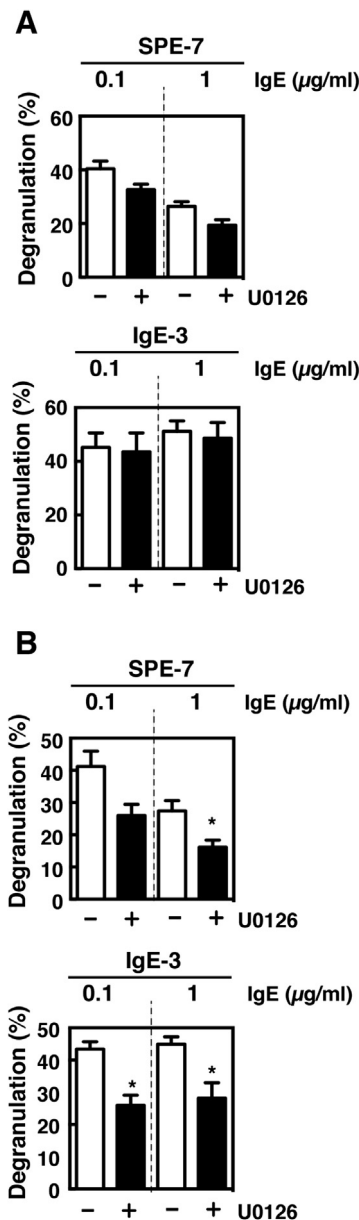


Fig. 6. Effects of U0126 on antigen-induced degranulation. (A) BMMCs pre-incubated with or without 10 μM U0126 for 15 min were sensitized with the indicated concentrations of IgE (SPE-7 and IgE-3) for 24 h, and then stimulated with 30 ng/ml of the specific antigen. Values are presented as the means ± SEMs (n = 3). (B) BMMCs were sensitized with the indicated concentrations of IgE (SPE-7 and IgE-3) for 24 h, and washed to remove unbound IgE. The cells were incubated with 10 μM U0126 for 15 min, and then stimulated with 30 ng/ml of the specific antigen. Values are presented as the means ± SEMs (n = 3). Values with **p* < 0.05 (vs. without U0126) are regarded as significant. The levels of degranulation were determined by measuring β-hexosaminidase activity.

suppress degranulation of BMMCs at high concentrations mainly through blocking the enzymatic activity of PI3K, p110δ subunit [24], it is plausible that inhibition of PI3K should be involved in the SP600125-mediated inhibition of antigen-induced degranulation. In a serum-transfer murine arthritis model, joint mast cell degranulation is less efficient in *Mapk8*^{-/-} mice [34]. Expression of JNK1 in mast cells was found to be required for pathogenesis of arthritis and IL-1β release, indicating the critical roles of JNK1 in activation of mast cells, although it remains to be clarified whether JNK1 directly regulates degranulation.

In summary, we demonstrated that differences of sensitizing IgE clones could affect the nature of antigen-induced activation of

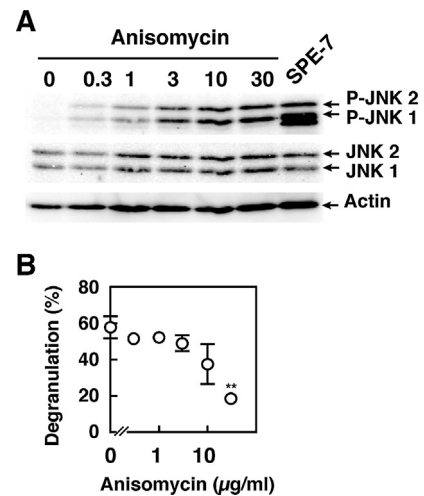


Fig. 7. Suppressive effects on antigen-induced degranulation of anisomycin treated before sensitization with IgE. (A) BMMCs were treated with the indicated concentrations (μg/ml) of anisomycin or 1 μg/ml SPE-7 for 10 min. Phosphorylation of JNK was detected using the specific antibodies. (B) BMMCs were treated with the indicated concentrations of anisomycin for 10 min and then washed three times. Anisomycin-treated BMMCs were sensitized with 1 μg/ml of IgE-3 for 24 h and then stimulated with the antigen (30 ng/ml). Values are presented as the means ± SEMs (n = 3). Values with ***p* < 0.01 (vs. no anisomycin) are regarded as significant.

murine cultured mast cells and that activation of JNK on the onset of sensitization may be involved in suppression of the antigen-induced degranulation. Since JNK was found to be activated during stress and inflammatory responses, JNK-mediated suppression of mast cell activation may function as a negative feedback system operating during potent inflammatory responses.

Disclosure

No conflicts of interest, financial or otherwise, are declared by the author(s).

Acknowledgements

This work was supported in part by JSPS KAKENHI Grant Number 19590091, 21790100 and 2667029.

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