



Inhibitory effects of parthenolide on antigen-induced microtubule formation and degranulation in mast cells

Naoko Miyata^{a,b}, Yasuhiro Gon^{a,*}, Satoshi Nunomura^a, Daisuke Endo^{a,b},
Kyoko Yamashita^{a,b}, Ken Matsumoto^b, Shu Hashimoto^b, Chisei Ra^a

^a Division of Molecular Cell Immunology and Allergology, Department of Advanced Medical Research Center, Nihon University School of Medicine, Japan

^b Division of Respiratory Medicine, Department of Internal Medicine, Nihon University School of Medicine, 30-1 Oyaguchikami-cho, Itabashi-ku, Tokyo 173-8610, Japan

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Abstract

Pharmacological modulation of IgE-mediated mast cell activation is important to the development of anti-allergic reagents. In this study, we investigated the effects of parthenolide (PTL) on high-affinity IgE receptor (FcεRI)-induced degranulation in mast cells. PTL dose-dependently inhibited degranulation induced by IgE-antigen stimulation in RBL-2H3 cells and BMDCs. Although PTL is a potent NF-κB inhibitor by targeting IκB kinase complex, NF-κB inhibition by other IκB kinase inhibitors did not inhibit degranulation in mast cells. IgE-antigen-induced microtubule formation is well known to be critical for degranulation in mast cells. Immunocytochemical study with anti-α-tubulin antibody revealed that PTL significantly inhibited IgE-antigen-induced microtubule formation. However, PTL, as well as nocodazol, had no significant effects on degranulation in the fyn-deficient BMDCs, suggesting that inhibitory effects of PTL in the microtubule formation are fyn dependent. We further demonstrated that *in vivo* administration of PTL in mice strongly inhibited passive cutaneous anaphylaxis reaction. The present study provides a possibility to develop potent reagents against mast cell activation based on an inhibition of microtubule formation.

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Abbreviations: Ab, antibody; BMDC, bone marrow-derived mast cell; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; DNP, dinitrophenyl; FcεRI, high-affinity receptor for IgE; ERK, extracellular signal-regulated kinase; FITC, fluorescein isothiocyanate; cPKC, conventional PKC; IκB, inhibitor of NF-κB; IKK, IκB kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, Nuclear Factor-κB; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; PFA, paraformaldehyde; PTL, parthenolide; RBL, rat basophilic leukemia; TNP, trinitrophenol; WT, wild-type.

* Corresponding author.

E-mail address: gon@med.nihon-u.ac.jp (Y. Gon).

1. Introduction

Aggregation of the high-affinity IgE receptor (FcεRI) on mast cells triggers degranulation with release of inflammatory mediators, such as histamine, proteases, inflammatory cytokines and lipid mediators, which are significantly involved in pathogenesis of allergic diseases such as bronchial asthma, atopic dermatitis and hay fever, etc. [1–3]. Therefore, pharmacological modulation of mast cell degranulation is a critical area for the development of anti-allergic reagents.

Parthenolide (PTL), a sesquiterpene lactone isolated from the herb feverfew (*Tanacetum parthenium*), is known to exhibit anti-inflammatory properties and is used clinically for treatment of migraine [4,5]. PTL has been shown to inhibit activity of upstream kinase responsible for phosphorylating I κ B proteins, known as I κ B kinase- β [6], and also been reported to inhibit LPS-induced ERK1/2 MAP kinase activation in rat neural cells and LPS-induced p38 MAP kinase activation in human dendritic cells [7]. It is suggested that these effects of PTL contribute to its anti-inflammatory properties.

Although the mechanisms underlying anti-inflammatory properties of PTL are reasonably well documented, biological activity of PTL against allergic reactions remains poorly understood. In the present study, we investigated biological effects and further elucidated mechanisms of the inhibitory effects of PTL on mast cell degranulation induced by IgE-antigen stimulation. We found that PTL prevents the mast cell degranulation *in vitro* by a specific inhibition of microtubule formation induced by IgE-antigen stimulation. We also confirmed that *in vivo* treatment with PTL attenuated IgE-mediated passive cutaneous anaphylaxis reaction in mice. Our findings may provide further information for the development of potent anti-allergic reagents based on a new mechanism interfering with mast cell degranulation.

2. Materials and methods

2.1. Reagents

Parthenolide (PTL) was purchased from Calbiochem (San Diego, CA, USA). Anti 2,4,6-trinitrophenol (TNP) IgE monoclonal antibody (mAb)

(clone IgE-3) and TNP-BSA (25 mol of TNP per 1 mol of BSA) were purchased from BD PharMingen Japan (Tokyo, Japan) and LSL (Tokyo, Japan), respectively. Anti- α -tubulin mAb, calcium ionophore (A23187), paclitaxel and nocodazol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant IL-3 (rIL-3), anti-phospho cPKCs (PKC-pan), ERK, p38, JNK Ab, anti-phosphotyrosine (PY20) were purchased from Calbiochem (San Diego, CA, USA), Cell Signaling Biotechnology (Beverly, MA, USA), Amersham Life Science, (Birmingham, UK), respectively.

2.2. Cell culture

Bone marrow-derived murine mast cells (BMMCs) were prepared from C57BL/6J mice as previously described [8]. Fyn-deficient BMMCs were obtained from Fyn-deficient mice (BRC No. 01000), which were kindly provided by Dr. T. Yagi (Osaka University) and RIKEN BRC, with the support of National BioResource Project of Ministry of Education, Culture, Sports, Science and Technology, Japan [9]. The Animal Care and Use Committee of Nihon University approved all animal experiments. Briefly, cells were cultured in a RPMI 1640 medium (Sigma) supplemented with 10% fetal bovine serum (FBS) (SAFC Biosciences, Lenexa, KS, USA) and 5 ng/ml rIL-3 at 37 °C, 5% CO₂, in a humidified incubator. RBL-2H3 cells obtained from the National Institute of Health Sciences (Japan Collection of Research Biosources, JCRB) (Cell No. JCRB0023) were grown in Dulbecco's minimal essential medium (DMEM; Sigma) and supplemented with 10% FBS (SAFC Biosciences) at 37 °C, 5% CO₂, in a humidified incubator.

2.3. Degranulation assay

Degranulation was determined by β -hexosaminidase release. After 1 μ g/ml of anti-TNP IgE-sensitized RBL-2H3 cells (2×10^5 cells/ml) or BMMCs (2×10^5 cells/ml) were stimulated with the indicated dose

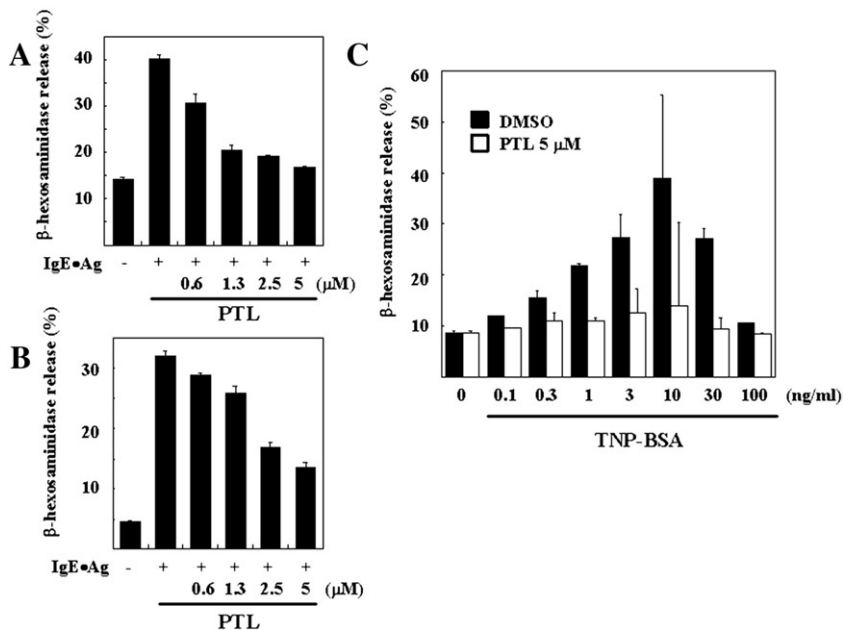


Figure 1 Effects of PTL on mast cell degranulation. (A). BMMCs (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with the indicated concentrations of PTL for 1 h, and then stimulated with 10 ng/ml TNP-BSA for 30 min. (B). RBL-2H3 cells (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with the indicated concentrations of PTL for 1 h, and then stimulated with 30 ng/ml TNP-BSA for 30 min. (C). BMMCs (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with 5 μ M of PTL or 0.1% DMSO for 1 h, and then stimulated with the indicated concentrations of TNP-BSA as indicated for 30 min. Degranulation was monitored by β -hexosaminidase activity released into culture supernatants in all experiments. Data are means $\pm$ SD of three measurements in a representative of three different experiments with similar results.

of TNP-BSA at 37 °C for 30 min, the supernatants were collected and β -hexosaminidase activity in the supernatants was determined spectrophotometrically as previously described [8]. The cells were lysed with 0.1% Triton X-100, and the extract was analyzed for the total β -hexosaminidase activity. The percentage of β -hexosaminidase released into the supernatant was calculated using the following method: $100 \times (\text{supernatant OD value of the stimulated cells}) / (\text{total cell lysate OD value})$.

2.4. Immunocytochemistry

Separate aliquots of 5×10^5 RBL-2H3 cells were cultured on microcoverglass (Matsunami, Tokyo, Japan) in a 6-well plate (Coster, NY, USA), and the cells were sensitized with 1 $\mu\text{g}/\text{ml}$ anti-TNP IgE for 6 h. The cells were then pretreated with 5 μM PTL for 60 min. After pretreatment, the cells were washed twice with calcium- and magnesium-free Phosphate Buffered Saline [PBS(-)]. The cells were then stimulated with 100 ng/ml TNP-BSA for 5 min at 37 °C and fixed with 4% paraformaldehyde (PFA) for 30 min at 37 °C. Anti- α -tubulin mAb (B5-1-2; Sigma-Aldrich) was used as a primary antibody, and Alexa 488-conjugated anti-mouse IgG was used as a secondary antibody. Staining was performed for 30 min in the dark. The slides were then washed with PBS(-) and coverslips were mounted with Gel/mount (Biomed, Foster City, CA). Microscopic analyses were performed using a Fluoview system (Olympus, Tokyo, Japan).

2.5. Western blot

The stimulated cells were washed twice with ice-cold PBS and lysed in Tris Buffer Saline containing 1% Nonidet P-40, 60 mM octyl- β -glucoside, 2 mM phenylmethylsulfonylfluoride (PMSF), 10 $\mu\text{g}/\text{ml}$ aprotinin, 2 $\mu\text{g}/\text{ml}$ leupeptin and pepstatin A, 50 mM NaF and 1 mM sodium orthovanadate, for 30 min on ice. The lysates were centrifuged for 15 min at $14,000 \times g$. The samples were then boiled, separated on 10% SDS-PAGE, and transferred onto Immobilon-P membrane (Millipore, Bedford, MA, USA). The membrane was incubated with primary antibodies and the appropriate secondary horseradish peroxidase-conjugated antibodies. Signals were detected by enhanced chemiluminescence (GE Health Care, Little Chalfont, UK).

2.6. Transfection and luciferase reporter assays

A NF- κ B-luciferase plasmid, containing five copies of the consensus NF- κ B site linked to a minimal promoter-luciferase reporter gene, was purchased from Stratagene (La Jolla, CA). RBL-2H3 cells were cultured at $0.5 \times 10^6/\text{ml}$ in 12-well plates (1 ml/well) for 16–20 h and were transfected with 1 mg of NF- κ B-luciferase plasmid by using Fugene6 (Roche Diagnostic Systems, Somerville, NJ). Cells were allowed to recover for 24 h and then were sensitized with anti-TNP IgE for 3 h. Cells were stimulated with TNP-BSA for 4 h. Cell lysates were prepared and were assayed by the Promega Luciferase Assay System (Promega Biotech, Madison, WI), used in accordance with the manufacturer's instructions. In some experiments, pRL-Tk (Promega) was co-transfected as an internal control to normalize differences in the transfection efficiency. The lysates from these cells were quantified for luciferase using the Dual-Luciferase Reporter Assay System (Promega).

2.7. Passive cutaneous anaphylaxis (PCA) reaction

A total of 0.5 μg of IgE was injected subcutaneously (s.c.) into the left ears of BALB/c mice 24 h before the antigen challenge. After IgE-sensitization, either vehicle or PTL was injected intraperitoneally (i.p.) into the mice at the concentration indicated 150 min before the antigen challenge, and the mice were tested with an

intravenous injection (i.v.) of 250 μg of polyvalent dinitrophenyl-BSA (DNP-BSA; LSL) in 250 μl of saline with Evans blue dye (5 mg/ml) (Sigma-Aldrich). Extravasation of Evans blue in the ear was monitored for 30 min, the mice were then euthanized, both ears were dissected, and the Evans blue dye was extracted in 700 μl of formamide overnight at 65 °C. The optimal absorbance of Evans blue-extracted formamide was then measured at a 620 nm wavelength. We obtained approval from the Animal Research Committee at Nihon University for all animal experiments performed.

2.8. Statistical analysis

The statistical significance was analyzed by using an analysis of variance (ANOVA). P -values < 0.05 were considered significant. When statistical significance was reached, post hoc tests (Fischer's protected least significant difference, and Scheffe's F test) were performed.

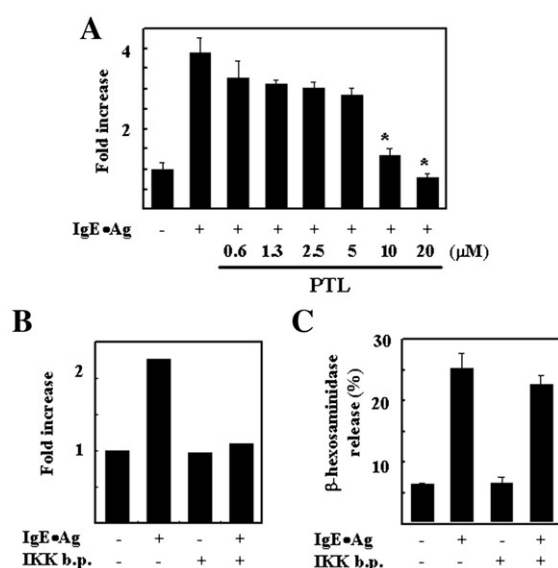


Figure 2 Effects of PTL on NF- κ B activation. (A). RBL-2H3 cells (2×10^5 cells/ml) were transiently transfected with NF- κ B luciferase reporter plasmids. The cells were sensitized with 1 $\mu\text{g}/\text{ml}$ of anti-TNP IgE and stimulated with 30 ng/ml TNP-BSA for 4 h after preincubation with PTL at the indicated concentrations for 1 h. Luciferase activity in the cytoplasmic extracts was measured, and the results are given as the fold increase of mean luciferase light units with reference to unstimulated cells. The results are the mean SD of three different experiments. * $p < 0.01$ compared with IgE-antigen-stimulated cells. (B). RBL-2H3 cells (2×10^5 cells/ml) were transiently transfected with NF- κ B luciferase reporter plasmid. The cells were sensitized with 1 $\mu\text{g}/\text{ml}$ of anti-TNP IgE and stimulated with 30 ng/ml of TNP-BSA for 4 h after preincubation with 100 μM IKK blocking peptide and/or 5 μM PTL, and luciferase activity was measured. Data are means $\pm$ SD of three measurements in a representative of three experiments with similar results. (C). RBL-2H3 cells (2×10^5 cells/ml) were sensitized with 1 $\mu\text{g}/\text{ml}$ of anti-TNP IgE overnight. The cells were pretreated with 100 μM IKK blocking peptide, and then stimulated with 30 ng/ml TNP-BSA for 30 min. Degranulation was monitored by β -hexosaminidase activity released into culture supernatants. Data are means $\pm$ SD of three measurements in a representative of three different experiments with similar results.

3. Results

3.1. Parthenolide inhibits mast cell degranulation

To examine effects of PTL on mast cell degranulation, sensitized BMMCs and RBL-2H3 cells were pretreated with PTL for 1 h before IgE-antigen stimulation. Mast cell degranulation was evaluated by β -hexosaminidase release assay. Pretreatment with PTL did not affect cellular viability, as assessed by annexinV/PI staining (data not shown). As shown in Fig. 1, pretreatment of BMMCs (Fig. 1A) and RBL-2H3 cells (Fig. 1B) with PTL (0.6 μ M–5 μ M) markedly reduced the degranulation induced by IgE-antigen stimulation in a dose-dependent manner. Almost the same level of inhibitory effects of the reagent was observed at the various concentrations of antigen (0.1–100 ng/ml TNP-BSA) in BMMCs (Fig. 1C).

3.2. Inhibition of mast cell degranulation by PTL is independent of NF- κ B

PTL has been reported to inhibit NF- κ B activity by targeting the I κ B kinase complex [6]. We studied a possibility that PTL might inhibit the degranulation through inhibition of NF- κ B. As shown in Fig. 2A, 10 and 20 μ M of PTL significantly inhibited NF- κ B, as previously reported in other cells [10,11]. PTL (5 μ M) did not exhibit any inhibitory effects on NF- κ B activity, whereas this concentration significantly inhibited IgE-antigen-induced degranulation (Fig. 1B). The treatment of a cell-permeable peptide inhibitor of the I κ B kinase complex significantly attenuated IgE-antigen-induced NF- κ B dependent luciferase gene

expression in RBL-2H3 cells (Fig. 2B), whereas this reagent had no effects on degranulation (Fig. 2C). Taken together, these results indicate that inhibitory effects of PTL on IgE-antigen-induced degranulation are independent of its inhibitory effects on NF- κ B activity.

3.3. PTL did not affect tyrosine phosphorylation of cPKCs and MAP kinases

We next investigated the effects of PTL on Fc ϵ RI-mediated intracellular signal transduction pathways in the IgE-antigen-stimulated mast cells. IgE-antigen stimulation induced rapid tyrosine phosphorylation of a variety of proteins in RBL-2H3 cells (Fig. 3A). PTL pretreatment did not affect tyrosine phosphorylation of these proteins after IgE-antigen stimulation (Fig. 3A). IgE-antigen stimulation induced MAP kinase and conventional PKCs (cPKCs) activation in RBL-2H3 cells (Fig. 3B). Pretreatment with PTL did not significantly influence the phosphorylation levels of MAP kinase and cPKCs in RBL-2H3 cells (Fig. 3B).

3.4. PTL inhibits microtubule formation

The formation of microtubules is well known to be crucial for IgE-antigen-induced degranulation [12] in mast cells, and PTL has been reported to exhibit tubulin/microtubule-interfering activity [13]. Based on this evidence, we hypothesized that PTL could interfere with Fc ϵ RI-induced microtubule formation, resulting in the attenuation of mast cell degranulation. We employed nocodazol as a microtubule-disrupting agent to test our hypothesis. Pretreatment with 5 μ M PTL for 60 min, 33 μ M nocodazole

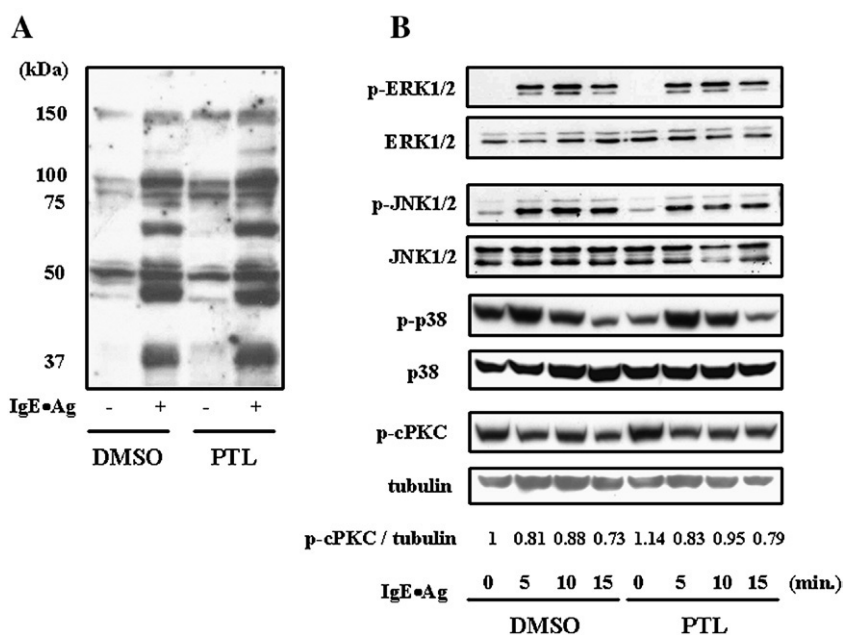


Figure 3 Effects of PTL on tyrosine phosphorylation, cPKCs, and MAP kinase activation. (A). RBL-2H3 cells (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with 5 μ M of PTL for 1 h, and then stimulated with 30 ng/ml TNP-BSA. Tyrosine phosphorylation was measured by Western blotting with an anti-phosphotyrosine Ab (4G10). The results presented are representative of three different experiments with similar results. (B). RBL-2H3 cells (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with 5 μ M of PTL for 1 h, and then stimulated with 30 ng/ml TNP-BSA. MAPKs and cPKCs phosphorylation were measured by Western blotting with anti-MAPKs phosphorylation specific Abs. The band intensity of phosphor-cPKC and α -tubulin was calculated with NIH Image J software. The results presented are representative of three different experiments with similar results.

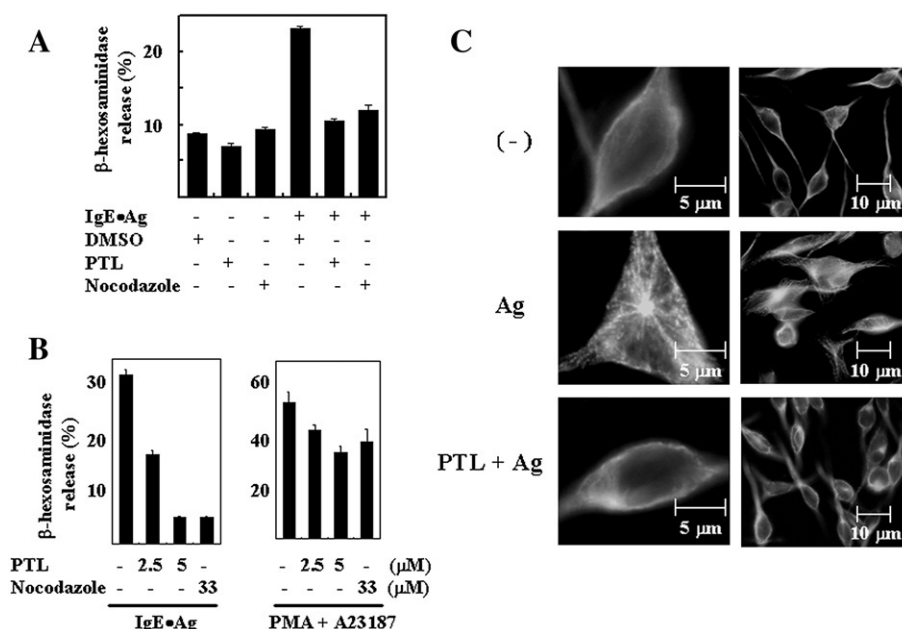


Figure 4 Effects of PTL on microtubule formation. (A) RBL-2H3 cells (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with 5 μ M PTL or 33 μ M nocodazole, and then stimulated with 30 ng/ml TNP-BSA. Data are means $\pm$ SD of three measurements in a representative of three different experiments with similar results. (B) RBL-2H3 cells (2×10^5 cells/ml) were pretreated with the indicated concentrations of PTL or nocodazole for 1 h, and then stimulated with PMA and A23187. Experimental values are given as means $\pm$ SD of triplicate assays. The results presented are representative of three different experiments with similar results. (C) RBL-2H3 cells (5×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with 5 μ M PTL or 0.1% DMSO for 1 h, and then stimulated with 30 ng/ml TNP-BSA in a Ca^{2+} -free medium. Cells were stained with anti- α -tubulin antibodies followed by Alexa 488-labeled secondary antibodies. Data are means $\pm$ SD of three measurements in a representative of three different experiments with similar results.

for 60 min resulted in inhibition of Fc ϵ RI-induced mast cell degranulation (Fig. 4A). In contrast, nocodazole and PTL exhibited only a small inhibitory effect on the PMA and calcium ionophore-induced mast cell degranulation (Fig. 4B). We next performed

immunostaining for α -tubulin to investigate effects of PTL on the microtubule formation in RBL-2H3 cells. In the resting state, microtubules were confined to the plasma membrane and the cytoplasm adjacent to nuclei, similar to a microtubule-

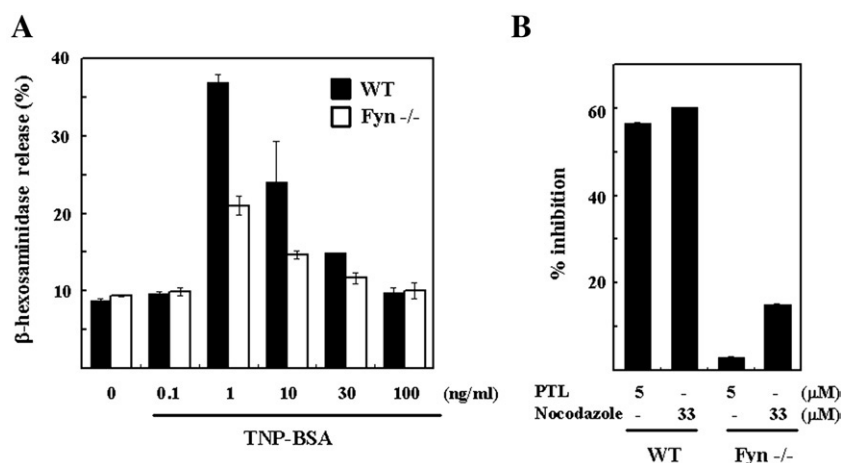


Figure 5 Effects of PTL on Fyn-deficient BMMCs. (A) WT or Fyn-deficient [Fyn(-/-)] BMMCs (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were stimulated with the indicated concentrations of TNP-BSA. Degranulation was monitored by β -hexosaminidase activity released into culture supernatants. Data are means $\pm$ SD of three measurements in a representative of three different experiments with similar results. (B) WT or Fyn(-/-) BMMCs (2×10^5 cells/ml) were sensitized with 1 μ g/ml of anti-TNP IgE overnight. The cells were pretreated with 5 μ M PTL for 1 h, and then stimulated with 1 ng/ml TNP-BSA. % inhibition = $100 - (\text{degranulation (\% in inhibitor treated cells)} / (\text{degranulation (\% in untreated cells)} \times 100)$. Data are means $\pm$ SD of three measurements in a representative of three different experiments with similar results.

organizing center (Fig. 4C, upper panels). After IgE-antigen stimulation, microtubules spread out in the direction to the plasma membrane (Fig. 4C, middle panels), and pretreatment with PTL prevented the microtubule formation (Fig. 4C, lower panels).

In the meantime, Fyn kinase has been reported to regulate IgE-antigen-induced microtubule formation. The loss of Fyn kinase in BMMCs (Fyn^{-/-} mice) diminished the degranulation by approximately 50% compared with wild-type (WT) BMMCs (Fig. 5A). We tested the effects of PTL on the FcεRI-induced degranulation in Fyn-deficient BMMCs. Unlike WT BMMCs, neither PTL nor nocodazol affected degranulation in Fyn-deficient BMMCs (Fig. 5B).

3.5. PTL inhibits PCA reaction

Since *in vivo* administration of PTL has been reported to prevent systemic inflammation in mice [14], we next examined the effects of PTL on *in vivo* allergic response. Intraperitoneal administration

of PTL at 150 min before antigen and dye injection substantially reduced PCA reaction (approximately 90% inhibition by a single dose of PTL at 10 mg/kg) as demonstrated in Fig. 6.

4. Discussion

FcεRI aggregation on mast cell leads to rapid rearrangement of the cytoskeleton, which is critical for mast cell activation. In the components of the cytoskeleton, microtubules are centrally involved in the FcεRI-mediated mast cell degranulation [12,15–17]. A recent study revealed that microtubule formation induced by FcεRI aggregation is required for the translocation of intracellular granules to the plasma membrane, which is the crucial step for IgE-antigen-induced degranulation [12]. As shown in Fig. 1, we found that PTL significantly inhibited the IgE-antigen-induced mast cell degranulation. Based on the previous reports that PTL has interfered with microtubule assembly in a cell-free system [13], we hypothesized that this interference activity is involved in the effects of PTL on mast cell degranulation. In the present study, we compared the inhibitory property of PTL on mast cell degranulation with that of other reagents which interfered with microtubule formation, a microtubule-disrupting agent, nocodazol, and found it reduced the IgE-antigen-induced degranulation of mast cells. Unlike FcεRI-mediated degranulation, nocodazol exhibited only marginal effects on PMA and calcium ionophore-induced mast cell degranulation, which indicates this stimulation may be microtubule independent. PTL also showed little effects on the PMA and calcium ionophore-induced degranulation, supporting that PTL interfered with FcεRI-mediated microtubule formation in a similar fashion of nocodazol. To further clarify this issue, we performed immunocytochemistry to visualize microtubule formation in RBL-2H3 cells, which revealed that IgE-antigen stimulation led to microtubule formation in the cytosol, and that PTL markedly reduced the microtubule formation.

In this study, we could not exclude the possibility that PTL indirectly affects microtubule formation via interference with FcεRI signaling molecules. Although a number of studies have shown that PTL is a potent inhibitor of NF-κB [6], there are few reports regarding the involvement of NF-κB activation in mast cell degranulation. We observed here that treatment with more than 10 μM PTL inhibited both the IgE-antigen-induced NF-κB activation and degranulation in RBL-2H3 cells, whereas pretreatment with 0.6–5 μM of PTL attenuated the degranulation, but not the NF-κB activation. In addition, inhibition of NF-κB activation by an IκB kinase blocking peptide [11] completely abolished the IgE-antigen-induced NF-κB activation, but with no effects on the mast cell degranulation. These findings suggest that PTL inhibits the IgE-antigen-induced mast cell degranulation by a NF-κB independent mechanism. We next examined whether PTL has effects on FcεRI-mediated early signaling molecules other than NF-κB, that is, tyrosine phosphorylation of a variety of proteins, p38, JNK1/2, ERK1/2, and cPKCs. However, no effects of PTL were observed on these signaling molecules, suggesting that PTL could directly interfere with microtubule formation in mast cells.

It was reported that Fyn-deficient BMMCs failed to migrate along Ag gradient [18], and that they were defective in

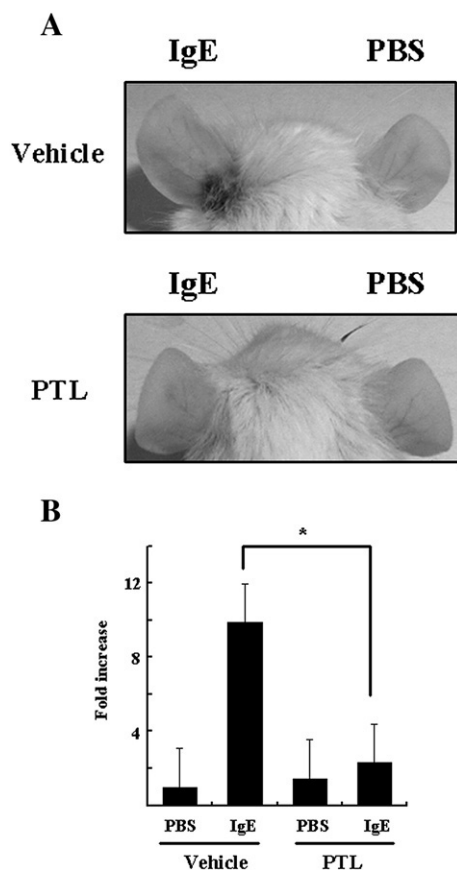


Figure 6 Effects of PTL on the PCA reaction. The left ears were intradermally injected with 0.5 μg anti-DNP IgE, whereas the right ears were intradermally injected with an equal amount of PBS. 24 h later, mice were injected intraperitoneally with DMSO or PTL (10 mg/kg). After 150 min, mice ($n=9$; each group) were intravenously injected with DNP-BSA and 1% Evans blue dye. The mice were evaluated for dye extravasation at 30 min after i.v. injection (A). The ears were cut off and dried at 65 °C for 24 h, and then incubated with formamide for 24 h. Evans blue dye was measured by a spectrophotometer with 620 nm (B). * $p<0.01$ compared with IgE treated control mice.

production of inflammatory mediators [19], and degranulation [20]. In addition, Fyn kinase has been shown to regulate the IgE-antigen-induced formation of microtubules [12]. In this study, we found that neither PTL nor nocodazol affected degranulation in Fyn-deficient mast cells, and concluded that the IgE-antigen-induced microtubule formation was dependent on Fyn kinase, and that PTL interfered with Fyn-dependent microtubule formation.

Administration of PTL into mice has been reported to prevent LPS-induced systemic inflammation [14]. In the present study, we found that PTL strongly inhibited the immediate-type allergic reaction, PCA reaction, in mice. No serious side effects have been reported in several clinical trials of PTL extracted from feverfew [4,5]. Of course, further experimental work is required to understand the actual benefit and risk of this reagent as an anti-allergic reagent.

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