

Original Research

Inhibitory effect of hot-water extract of quince (*Cydonia oblonga*) on immunoglobulin E-dependent late-phase immune reactions of mast cells

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Concise title: Effect of Quince on Late-phase Reactions of Mast Cells

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Abbreviations: Ag, antigen; BMMC, bone marrow-derived mast cell; COX, cyclooxygenase; DNP, dinitrophenyl; ELISA, enzyme-linked immunosorbent assay; FcεRI, high-affinity IgE receptor; FITC, fluorescein isothiocyanate; HW, hot-water extract; IgE, immunoglobulin E; IL, interleukin; LT, leukotriene; NSAID, nonsteroidal anti-inflammatory drug; PE, phycoerythrin; PG, prostaglandin; RT-PCR, reverse transcription-polymerase chain reaction; SD, standard deviation; TNF, tumor necrosis factor.

Abstract

We evaluated the effect of a crude hot-water extract (HW) of quince (*Cydonia oblonga* Miller) fruit on immunoglobulin E (IgE)-dependent late-phase immune reactions of mast cells using *in vitro* system. Mast cell-like RBL-2H3 cells were treated with quince HW and late-phase reaction was then induced by stimulation with IgE + Antigen (Ag). Quince HW reduced the elevation of interleukin (IL)-13 and tumor necrosis factor- α expression level. Furthermore, quince HW suppressed these cytokine expressions of mouse bone marrow-derived mast cells (BMMCs), a normal mast cell model. Leukotriene C₄ and prostaglandin D₂ production in BMMCs after 1 and 6 h of stimulation, respectively, were also reduced by treating the cells with quince HW. We found that the induction of intracellular cyclooxygenase (COX)-2 expression but not COX-1 expression in BMMCs was reduced by quince HW. These results suggest that quince HW has an inhibitory effect on broad range of the late-phase immune reactions of mast cells.

Keywords: quince; mast cell; late-phase reaction; leukotriene C₄; prostaglandin D₂; cyclooxygenase

1. Introduction

Type I allergy is an immediate-type inflammatory disease characterized by immunoglobulin E (IgE)-induced detrimental reactions of the immune system caused by environmental substances. Allergic reactions such as asthma, eczema, and, rhinitis have increased in recent years as a result of the modern industrial and hygienic ways of life in industrial countries. The symptoms of immediate-type allergies are caused by the action of mast cells and basophils (Stone et al. 2010). These cells are hematopoietic cell-derived immunocompetent cells that express of high-affinity immunoglobulin E (IgE) receptor (FcεRI) on their surface. Cross-linking of FcεRI by attaching IgE with allergen-derived multivalent antigen (Ag) triggers biochemical cascades that lead to degranulation, that is, the exocytotic release of granule-stored chemical mediators (Metzger 1978). The resultant inflammation caused by these mediators within minutes of exposure to Ag is termed “early-phase” in immediate-type allergic reactions (Williams and Galli 2000).

On the other hand, recent studies have focused on the subsequent the “late-phase” immune reactions that occur several hours after the exposure to Ag (Metz et al. 2007). In late-phase reactions, inflammatory cytokines, including interleukin (IL)-13 and tumor necrosis factor (TNF)- α , are synthesized (Gordon and Galli 1990, Gordon and Galli 1991, Burd et al. 1995). These cytokines contribute to the development of allergic symptoms through the mechanisms of class switching for IgE production from plasma cells (Cameron et al. 2000) and activation of phospholipase A₂ (Kang et al. 2008). The release of arachidonic acids by the action of activated phospholipase A₂ in response to the degranulation signalling further leads to the formation of metabolites, including leukotrienes (LTs) and prostaglandins (PGs) (Seeds and Bass 1999). These metabolites increase vascular permeability and initiates smooth muscle contraction (Dahlén et al. 1981, Dahlén et al. 1983, Giles and Leff 1988). Not only do these proinflammatory substances have the physiological functions, but also they also recruit several immune cells such as eosinophils, macrophages, T cells, and natural killer T cells to the inflammatory site, resulting in progression of the morbidity and severity of inflammatory allergic symptoms (Pawankar et al. 2003). Therefore, approaches designed to alleviate the onset of late-phase reactions of mast cells are considered to be effective in preventing immediate-type allergic symptom.

Recently, we reported the anti-allergic effect of a crude hot-water extract (HW) of quince on immediate allergic model NC/Nga mice (Shinomiya et al. 2009). Quince HW notably alleviated the

severity of atopic dermatitis-like skin lesions with degranulation of serum IgE. This study indicates that quince HW has some suppressive effects on immediate-type allergic reaction including the physiological function of mast cells.

In this study, we investigated the effect of quince HW on the IgE- and Ag-induced elevation of inflammatory cytokine expressions and production of lipid mediators in mast cells.

2. Materials and methods

2.1. Preparation of quince HW

Quince HW was prepared as described previously (Shinomiya et al. 2009). In brief, the peel, pulp and seeds of quince fruits were cut into pieces, and then boiled in hot water for 1 h. After filtering the extract through a layer of cloth, the filtrate was centrifuged at $5,000 \times g$ for 30 min. The resulting supernatant was concentrated to 450 ml in a rotary evaporator (RE 400A-W; Yamato, Tokyo, Japan) at 40°C, and freeze-dried to obtain quince HW.

2.2. Cells

The rat basophilic leukemia RBL-2H3 cell line was purchased from the Health Science Research Resources Bank (Osaka, Japan). The cells were maintained in complete RPMI1640 medium (Nissui Pharmaceutical, Tokyo, Japan) containing 10% heat-inactivated fetal bovine serum (FBS; Equitech-Bio, Kerrville, TX, USA), 2 mM L-glutamine (Invitrogen Life Technologies, Carlsbad, CA, USA), 100 IU/ml of penicillin, and 100 µg/ml of streptomycin in a humidified atmosphere of 5% CO₂/95% air at 37°C.

2.3. Mice

Specific pathogen-free DBA/2 Cr mice aged eight weeks were purchased from Japan SLC (Shizuoka, Japan) and housed at 23°C ± 3°C under a 12-h light/dark cycle. The mice were used at 6–8 weeks of age. All the animal protocols used in this study were approved by the Committee for Animal Experiments of Shinshu University.

2.4. Preparation and cultivation of mouse bone marrow-derived mast cells (BMMCs)

BMMCs were prepared from 6–8-week-old mice according to a previously described method (Lee *et al.*

2005). Mice were killed by cervical dislocation and their intact femurs were aseptically harvested. Bone marrow cells were obtained by repeatedly flushing the femurs with RPMI1640 medium containing 100 IU/mL penicillin and 100 µg/mL streptomycin. The cells thus obtained were washed twice with the same medium by centrifugation at $700 \times g$ for 10 min. The centrifuged cells were suspended in complete RPMI1640 medium supplemented with 10% FBS, 0.1 mM non-essential amino acids (Invitrogen), 5 ng/mL recombinant mouse IL-3 (Peprotech, Rocky Hill, NJ, USA), 50 µM 2-mercaptoethanol, 100 IU/mL penicillin, and 100 µg/mL streptomycin. They were then cultured at a density of 1×10^5 cells/mL in a humidified atmosphere of 5% CO₂/95% air at 37°C. After 4–5 weeks, the cells were subjected to flow cytometric analysis for the evaluation of cell surface FcεRI and c-Kit expression and to a β-hexosaminidase release assay, as described below.

2.5. Induction of IgE-mediated stimulation

RBL-2H3 cells or BMMCs (4×10^5 cells/ml) were treated with indicated concentration of quince HW for 24 h. The cells were harvested and washed twice with HEPES-Tyrode buffer (137 mM NaCl, 5.6 mM glucose, 2.7 mM KCl, 0.5 mM NaH₂PO₄, 1.0 mM CaCl₂ and 10 mM HEPES at pH 7.3) containing 0.1% bovine serum albumin. The washed cells were suspended in the same buffer in a centrifuge tube (BM Equipment, Tokyo, Japan) at a density of 1×10^7 cells/ml. Stimulation of the cells were conducted by using mouse monoclonal anti-dinitrophenyl IgE antibody (clone SPE-7; Sigma, St. Louis, MO, USA) as IgE and dinitrophenyl-labeled human serum albumin (Sigma) as Ag under indicated condition. After stimulation, the supernatant collected by centrifugation. The resultant pellet was washed twice with phosphate-buffered saline (pH7.2) and stored at –80°C until use.

In parallel with this assay, the growth and viability of quince HW-treated BMMCs were evaluated by counting the cells with a hemacytometer after staining with trypan blue.

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

RBL-2H3 cells and BMMCs (2×10^6 cells) were degranulated using 2 µg/ml IgE + 10 ng/ml Ag for the indicated time, and total RNA was extracted from them by using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. The extracted RNA (1 µg) was reverse-transcribed in a thermal cycler (PTC-200; MJ Research, Waltham, MA, USA) with 1 mM of each dNTP, 10 pmol/µl of oligo(dT)₁₈

primers, and 25 U/ μ l of M-MLV reverse transcriptase (Invitrogen) at 42°C for 50 min. The resulting cDNA was subjected to polymerase chain reaction with a SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) and 10 pmol/ μ l of the primers listed in Table 1. The PCR consisted of 1 cycle of preheating (95°C, 10 sec) and 45 cycles of denaturation (95°C, 5 sec), primer annealing (55°C, 10 sec), and extension (72°C, 20 sec) and was performed in a Thermal Cycler Dice Real Time System TP800 (Takara Bio) or a StepOnePlus Real Time PCR System (Life Technologies, Foster City, CA, USA). The PCR products were analyzed by the $\Delta\Delta$ Ct method using TP800 software (Takara Bio) or StepOne software (Life Technologies). The amount of the PCR product was normalized against the gene expression level of β -actin and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) for rat and mouse gene expressions, respectively.

2.7. Enzyme-linked immunosorbent assay (ELISA)

The level of histamine, LTC₄ and PGD₂ in supernatant was evaluated by a competitive ELISA. Supernatant of BMMCs was collected after stimulation with 2 μ g/ml IgE + 100 ng/ml Ag at indicated time from the cells by centrifugation at 10,000 \times g for 5 min. The resultant supernatant was subjected to ELISA using a Histamine EIA kit (SPI-Bio technology, Montigny-le-Bretonneux, France), LTC₄ EIA kit (Cayman Chemical, Ann Arbor, MI, USA) and PGD₂ EIA kit (Cayman Chemical) in accordance with the manufacturer's recommendation, respectively.

2.8. Flow cytometric analysis

The surface expression of c-Kit and Fc ϵ RI on BMMCs was determined by flow cytometric analysis. For Fc ϵ RI labeling, 1 \times 10⁶ BMMCs were washed twice with PBS and treated with 0.125 μ g/mL fluorescein isothiocyanate (FITC)-labeled anti-mouse Fc ϵ RI α Armenian hamster IgG (clone MAR-1; eBioscience, San Diego, CA, USA) and phycoerythrin (PE)-labeled anti-mouse c-Kit rat IgG (clone 2B8; eBioscience) at room temperature for 30 min. FITC-labeled Armenian hamster IgG isotype control (clone eBio299Arm; eBioscience) and PE-labeled rat IgG isotype control (clone eB149/10H5; eBioscience) were used as a negative control. After the cells were stained, they were washed with PBS (pH7.2) and analyzed using flow cytometer (FACSCalibur; BD Biosciences San Jose, CA, USA). All data were analyzed using the CellQuest Pro software (BD Biosciences).

2.9. Statistical analysis

The data were statistically analyzed using a two-tailed Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of quince HW on IgE-induced elevation of cytokine expressions in RBL-2H3 cells

The effects of quince HW treatment on the IgE + Ag-induced mRNA expressions in RBL-2H3 cells are shown in Fig. 1. After 2 h of stimulation with IgE + Ag, gene expression of IL-13 and TNF- α was markedly enhanced. The expression levels were then decreased after 4 h of stimulation (data not shown). Treatment with quince HW reduced the expression of IL-13 and TNF- α in a dose-dependent manner (Fig. 1). The reduction in IL-13 and TNF- α expression by quince HW treatment was statistically significant ($p < 0.01$ and $p < 0.05$, respectively).

3.2. Effect of quince HW on histamine release from BMMCs

After cultivating bone marrow-derived cells under IL-3-supplemented condition for 5 weeks, over 98% of the floating cells were confirmed as c-Kit⁺Fc ϵ RI⁺ cells (typical BMMCs) by flow cytometric analysis. On the basis of this result, the effect of quince HW on the function of BMMC was investigated.

Fig. 2 shows that histamine release from BMMCs after treatment with quince HW. The histamine level of degranulation supernatant after treatment with quince HW was dose-dependently reduced (Fig. 2A). Treatment with 500 μ g/ml of quince HW results in a statistically significant reduction in histamine release ($p < 0.05$). Under the treatment condition, quince HW at 500 μ g/ml concentration had no significant effect on the growth of cells even though the treatment was extended until 72 h (Fig. 2B). Viability of the cells (over 99%) was also unaffected by quince HW treatment (data not shown). In addition, after treatment with quince HW at 0, 50 and 500 μ g/ml for 24 h, the ratio of c-Kit⁺Fc ϵ RI⁺ cells was 98.7%, 98.4%, and 98.2% of total cells, respectively (Fig. 2C).

3.3. Effects of quince HW on IgE-induced elevation of cytokine expressions in BMMCs

To confirm that the effect of quince HW on IL-13 and TNF- α expression in normal mast cells, the relative

expression levels of both cytokine expressions in BMMCs after 2 h of stimulation was evaluated.

Treatment with quince HW dose-dependently reduced the elevation of IL-13 down to 28.0% and TNF- α down to 48.2% after 2 h, compared to those of the quince HW-untreated control (Fig. 3). The reduction in IL-13 and TNF- α expression by 500 μ g/ml of quince HW was statistically significant ($p < 0.01$ for IL-13 and $p < 0.05$ for TNF- α).

3.4. Effect of quince HW on IgE-induced lipid mediator release from BMMCs

The effects of quince HW treatment on the release of lipid mediator from BMMCs are shown in Fig. 4. The concentration of LTC₄ in degranulation supernatant harvested after 1 h of IgE + Ag stimulation was increased from 12.2 ng/ml to 14.5 ng/ml. In the presence of quince HW, the increment was suppressed to 13.3 ng/ml in a dose-dependent manner (Fig. 4A).

The concentration of PGD₂ was also increased from 196 pg/ml to 245 pg/ml in response to the stimulation with IgE + Ag (Fig. 4B). No significant change in the concentration of PGD₂ in the supernatant collected after 1 h was observed between quince HW-treated and quince HW-untreated cells. In contrast, the elevation of PGD₂ level of the supernatant harvested after 6 h of stimulation was observed. Quince HW significantly ($p < 0.001$) suppressed only the elevation after 6 h of IgE + Ag stimulation.

3.5. Effects of quince HW on IgE-dependent PGD₂ production and cyclooxygenase (COX) expression of BMMCs

The effects of quince HW on the enhancement of the COX-1 and COX-2 expression are shown in Fig. 5. The expression of only COX-2 was enhanced by stimulation with IgE + Ag. After treatment with quince HW, a significant decrease was observed in COX-2 elevation in a dose-dependent manner whereas the change in COX-1 expression was negligibly small.

4. Discussion

In this study, we focused the effects of quince HW on the IgE + Ag-induced late-phase immune reactions of mast cell using two model cells.

Fig. 1 shows the mRNA expressions of IL-13 and TNF- α in RBL-2H3 after treatment with and without quince HW. The expression of these cytokines was notably enhanced after 2 h of stimulation with

IgE + Ag and diminished after 4 h (data not shown). Throughout the period, quince HW suppressed the elevation of these cytokine expression after 2 h of stimulation. As reported by Shinomiya et al. (2009), treatment with quince HW at the concentration up to 50 µg/ml had no influence on growth and viability of RBL-2H3 cells.

RBL-2H3 cell is an established basophilic leukaemia cell line that is commonly used as a model of mast cells because this cell line has functional FcεRI and several characteristics of IgE-dependent immune reactions (Siraganian et al. 1982, Ortega et al. 1988, Funada et al. 2003). Our data obtained using RBL-2H3 cells suggest that quince HW has a directly suppressive effect on IgE-dependent functions of mast cells in the absence of other immunocompetent cells. However, recent studies have focused on the abnormal susceptibility and properties of this cell line, stating that it is not fully representative of mast cells and basophils (Passante et al. 2009). Therefore, we further investigated the inhibitory effects of quince HW using BMMCs, which are known as a model of normal mucosal-type mast cells (Razin et al. 1981, Schrader et al. 1981).

We confirmed that quince HW suppressed histamine release from BMMCs induced by stimulation with IgE + Ag (Fig. 2A). Treatment with quince HW even at 500 µg/ml showed no significant change in the proliferation and viability of BMMCs (Fig. 2B). In addition, assessment of the cell population on the basis of c-Kit and FcεRI expression revealed that the subset of the BMMCs' culture was also unchanged after treatment with 0-500 µg/ml quince HW (Fig. 2C). These results suggest that quince HW has inhibitory effects on degranulation of BMMCs without affecting the viability and population of the culture. On the basis of these results, we investigated the effect of quince HW on late-phase immune reactions of BMMCs.

Fig. 3 shows that quince HW significantly inhibited IgE- and Ag-induced IL-13 and TNF-α expression in BMMCs after 2 h of stimulation. In agreement with the observation in RBL-2H3 cells, the inhibitory effect of quince HW was effective on the reduction of IgE-dependent inflammatory cytokine expression in BMMCs.

BMMCs generate mainly PGD₂ and LTC₄ as arachidonic acid metabolites in response to the IgE-dependent stimulation (Razin et al. 1982). We investigated the effects of quince HW on lipid mediator release from BMMCs. As shown in Fig. 4A, quince HW reduced the level of LTC₄ in supernatant after 1 h of stimulation. LTC₄ is cysteinyl leukotriene produced via arachidonic acid cascade

activated by IgE-mediated stimulation. Degradation of LTC₄ level suggests that quince HW is effective for alleviating the release of LTC₄ from mast cells. In contrast, the level of the PGD₂ in the degranulation supernatant collected after 1 h of the stimulation was unchanged by treatment with quince HW (Fig. 4B). Further analysis revealed that the PGD₂ level in supernatant obtained after 6 h of the stimulation with IgE + Ag was significantly lowered by quince HW treatment. PGD₂ production by mast cells after stimulation with IgE + Ag was reported to occur in two distinct phases (Murakami et al. 1994, Kawata et al. 1995). The first phase production of PGD₂ is mediated by constantly expressed COX-1 using pre-released arachidonic acid within 30 min of stimulation. The second phase production was mediated by COX-2, an inducible form of COX (Hundley et al. 2001), and reached its maximum 4 to 8 h after activation. In order to confirm the reason of PGD₂ suppression, we investigated the effects of quince HW on the mRNA expression of COX-1 and COX-2. Fig. 5 shows that IgE-induced COX-2 expression was significantly suppressed by quince HW, whereas the expression of COX-1 was not affected. Therefore, the downregulation of COX-2 observed in quince HW-treated BMMCs may in part explain the reduced second phase production of PGD₂ from these cells. The inhibitors of COX used to reduce PG-derived pain and inflammatory symptoms are known as nonsteroidal anti-inflammatory drugs (NSAIDs). However, suppression of total COX by NSAIDs has been found to result in several side effects such as gastrointestinal ulcers and renal dysfunction by inhibiting physiological functions of COX-1 (Harirforoosh and Jamali 2009, Sostres et al. 2010). Therefore, selective suppression of COX-2 by quince HW is believed to be more beneficial for suppressing the induction of PGD₂. We confirmed the basal expression of COX-1 and COX-2 was unchanged by quince HW treatment (data not shown). Therefore, the suppressive effect of quince HW on the elevation of COX-2 expression may be useful for weakening the allergic symptoms caused by PGD₂.

Quince fruit has been reported to contain low-molecular-weight polyphenols such as 3-caffeoylquinic acids (chlorogenic acid) and 5-caffeoylquinic acids (Silva et al. 2004). Recently, 3-caffeoylquinic acids were reported to alleviate mast cell-dependent anaphylactic reaction induced by IgE + Ag (Qin et al. 2010). However, the effect of some of these polyphenols contained in quince fruit on late-phase reactions of mast cells has not been fully understood. Elucidation of the inhibitory substance in quince HW against late-phase reaction of mast cells is expected.

Taken together, we analyzed the effect of quince HW on late-phase immune reactions of mast

cells using *in vitro* model. Considering the role of inflammatory cytokines and lipid mediators, reduced late-phase immune reaction of mast cells provides possible explanation for suppressive effect of quince HW on allergic symptoms. Further study was desired for applying quince HW as a functional food to alleviate type I allergy.

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Table 1. Primer sequences used in this study

Target*	Sequence		Product size (bp)	Accession number
	Forward	Reverse		
Rat IL-13	5'-ACCAGAAGACTTCCCCTGTGC-3'	5'-TGCAACTGGAGATGTTGGTC-3'	105	L26913
Rat TNF- α	5'-ACGATGCTCAGAAACACACG-3'	5'-GAGACAGCCTGATCCACTCC-3'	168	BC107671
Rat β -actin	5'-GTCTGTACCACTGGCATTGTG-3'	5'-TCTCAGCTGTGGTGTGAAC-3'	180	BC063166
Mouse IL-13	5'-CAGCATGGTATGGAGTGTGG-3'	5'-AGGCCATGCAATATCCTCTG-3'	117	NM_008355
Mouse TNF- α	5'-TAGCCAGGAGGAGAACAGA-3'	5'-TTTTTCTGGAGGGAGATGTGG-3'	127	M13049
Mouse COX-2	5'-TTGGGGAGACCATGGTAGAG-3'	5'-CATTGATGGTGGCTGTTTTG-3'	231	M94967
Mouse GAPDH	5'-CACTGAGCATCTCCCTCAC-3'	5'-GTGGGTGCAGCGAACTTTAT-3'	111	BC096042

Figure Captions

Fig. 1. Effects of quince HW on cytokine expression in RBL-2H3 cells. The expression of all cytokines was analyzed after 2 h of stimulation with IgE + Ag. Control means no stimulus (without IgE + Ag). Data are represented as the relative mean value \pm standard deviation (SD) against the unstimulated control (n = 3). * p < 0.05 and ** p < 0.01 vs. quince HW-untreated cells.

Fig. 2. Effects of quince HW on histamine release, growth, and phenotype of BMMCs. (A) Histamine concentration in degranulation supernatant of BMMCs after stimulation with IgE + Ag. Control means no stimulus (without IgE + Ag). Data are represented as the mean value \pm SD (n = 3). * p < 0.05 vs. quince HW-untreated cells. (B) Proliferation of BMMCs in the presence of quince HW. Cells were analyzed after treatment with and without quince HW for 0–72 h. White, gray, and black circle represents the result in the presence of 0, 50, and 500 μ g/ml quince HW, respectively. Data are represented as the mean value \pm SD (n = 3). (C) Cell surface expression of c-Kit and Fc ϵ RI of BMMCs. Cells were analyzed after treatment with 0, 50, and 500 μ g/ml quince HW for 24 h. Negative control means the result after staining with isotype control antibodies (without treatment of quince HW). The numbers in the plots indicate the percentage of sorted cell population in each quadrant.

Fig. 3. Effects of quince HW on cytokine expression of BMMCs. The expression of all cytokines was analyzed after 2 h of stimulation with IgE + Ag. Control means no stimulus (without IgE + Ag). Data are represented as the relative mean value \pm SD against the unstimulated control (n = 3). * p < 0.05, ** p < 0.01 and *** p < 0.001 vs. quince HW-untreated cells.

Fig. 4. Effects of quince HW on the production of lipid mediators in BMMCs. (A) LTC₄ production in BMMCs after stimulation with IgE + Ag for 2 h. Control means no stimulus (without IgE + Ag). Data are represented as the relative mean value \pm SD against the unstimulated control (n = 3). * p < 0.05 vs. quince HW-untreated cells. (B) PGD₂ production in BMMCs after stimulation with IgE + Ag

for 1 h (grey dotted bar) and 6 h (black dotted bar). Control means no stimulus (without IgE + Ag).

Data are represented as the relative mean value \pm SD against the unstimulated control (n = 3). *** p < 0.001 vs. quince HW-untreated cells.

Fig. 5. Effects of quince HW on COX-1 and COX-2 expression of BMMCs. Gene expression was

analyzed after stimulation with IgE + Ag for 2 h. Control means no stimulus (without IgE + Ag).

Data are represented as the relative mean value \pm SD against the unstimulated control (n = 3). ** p < 0.01 vs. quince HW-untreated cells.

Fig. 1

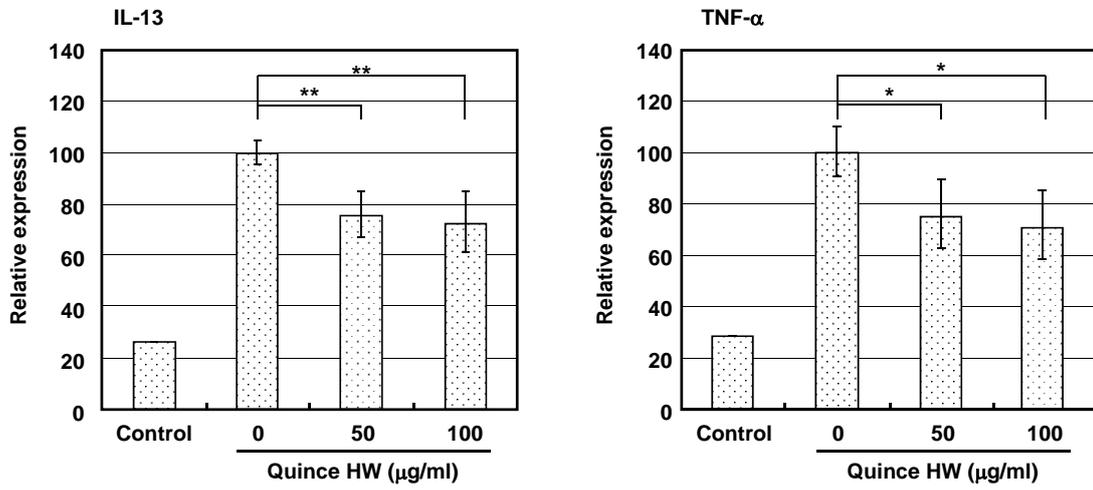


Fig. 2

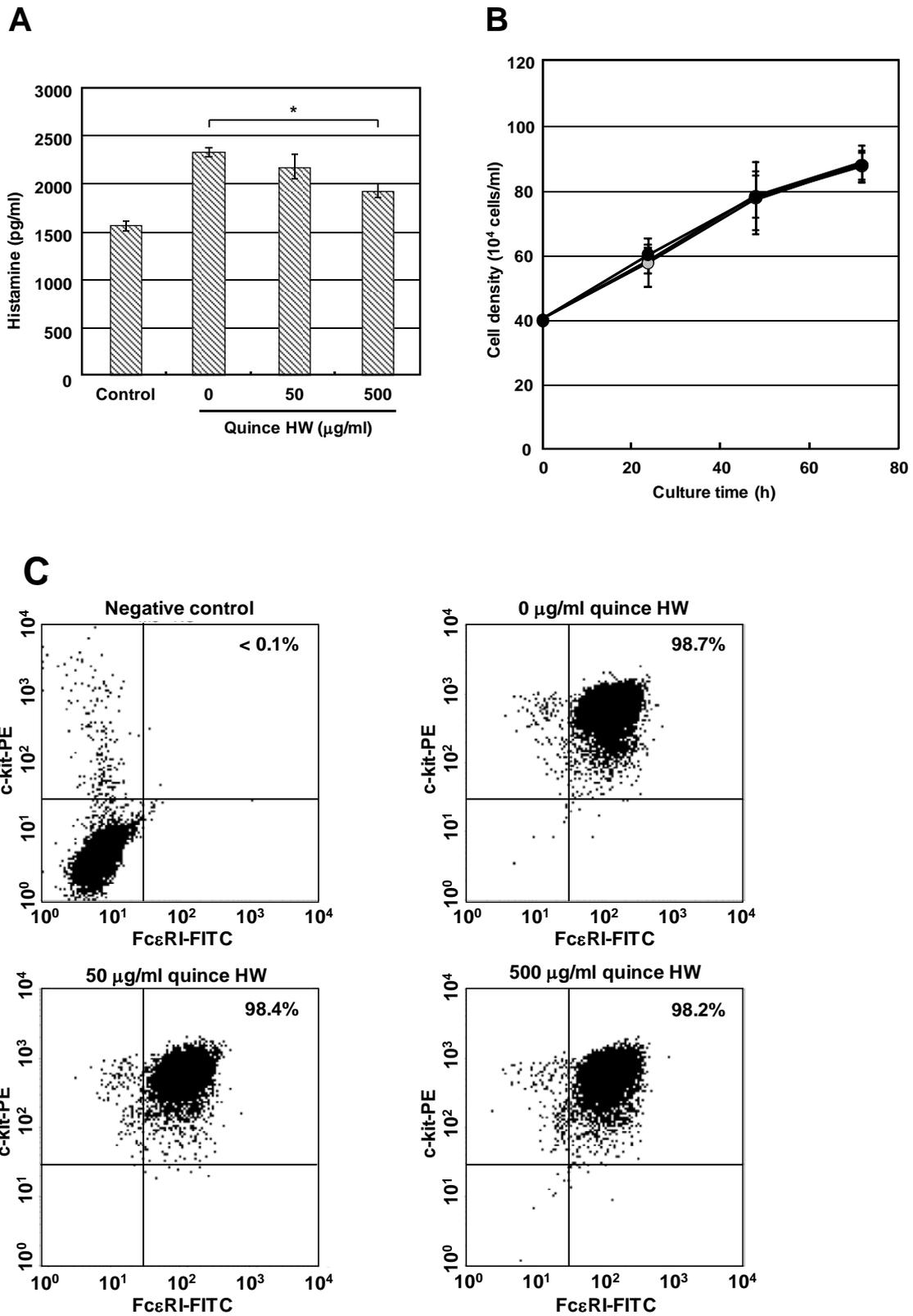


Fig. 3

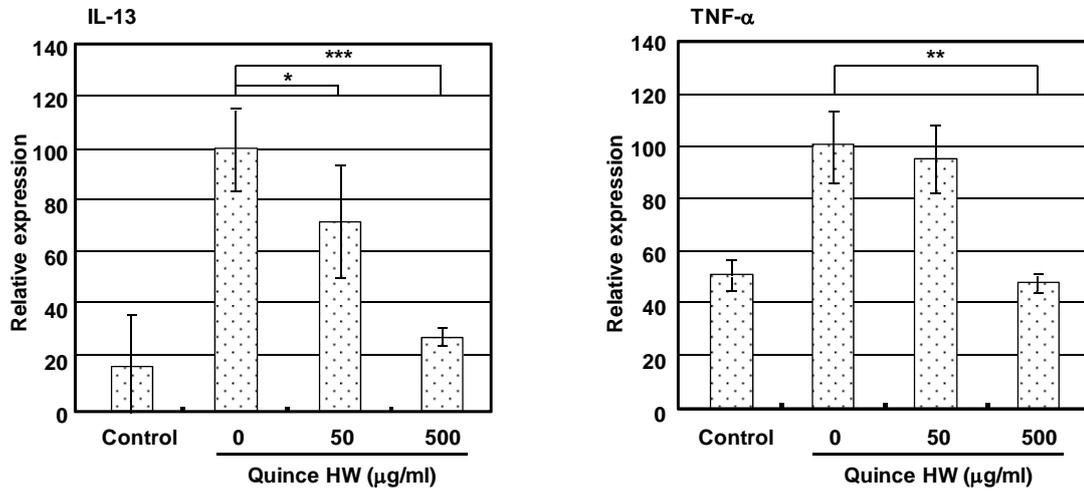


Fig. 4

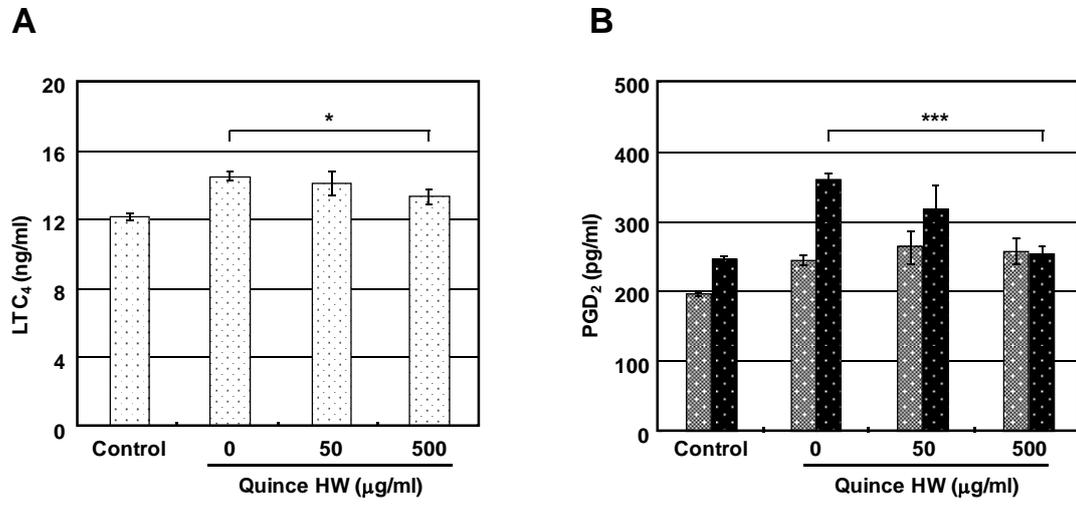


Fig. 5

