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Suppressive Activity of Quercetin on Periostin Functions *In Vitro*

SHINJI IRIE[1], MISAKO KASHIWABARA[1], ASAKO YAMADA[1] and KAZUHITO ASANO[2]

[1]Graduate School of Nursing and Rehabilitation Sciences, and [2]Division of Physiology, School of Nursing and Rehabilitation Sciences, Showa University, Yokohama, Japan

Abstract. *Periostin, a 90-kDa extracellular matrix protein, has been attracting attention as a novel biomarker of airway inflammatory diseases such as allergic rhinitis (AR) and asthma. Although oral administration of quercetin to patients with AR can favorably modify the clinical condition of this disease, the influence of quercetin on periostin functions is not well understood. The present study was, therefore, undertaken to examine the influence of quercetin on the production of both periostin and periostin-induced eosinophil chemoattractants from human nasal epithelial cells (HNEpC) in vitro. HNEpC were stimulated with 15.0 ng/ml interleukin (IL)-4 in the absence or presence of quercetin for 72 h. Periostin levels in the culture supernatants were measured using enzyme-linked immunosorbent assay (ELISA). Addition of 4.0 μM quercetin into cell cultures suppressed periostin production from HNEpC that was induced by IL-4 stimulation through inhibitation of signal transducer and activator of transcription 6 (STAT6) activation. We then examined whether quercetin could inhibit production of the periostin-induced eosinophil chemoattractants, regulated on activation, normal T-cell expressed and secreted (RANTES) and eotaxin, from HNEpC. HNEpC were stimulated with 2.0 ng/ml periostin in the absence or presence of quercetin for 72 h. RANTES and eotaxin levels in culture supernatants were examined using ELISA. Treatment of HNEpC with quercetin at a concentration of 4.0 μM suppressed the ability of cells to produce RANTES and eotaxin. This suppression was mediated through suppression of activation of the transcription factor nuclear factor-kappa B (NF-κB) p65, as measured using ELISA, and of chemokine mRNA expression, as measured using reverse transcriptase-polymerase chain reaction (RT-PCR). These results strongly suggest that quercetin suppresses the production of both periostin and periostin-induced eosinophil chemoattractants from HNEpC and results in improvement of the clinical condition of AR.*

Correspondence to: Shinji Irie, Graduate School of Nursing and Rehabilitation Sciences, Showa University Graduate School, 1865 Touka-Ichiba, Midori-Ku, Yokohama 226-8555, Japan. Tel: +81 459856552, Fax: +81 459856552, e-mail: irie@nr.showa-u.ac.jp

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Allergic rhinitis (AR) is a hypersensitivity response to specific allergens in the nasal mucosa. Aeroallergen exposure in sensitized individuals with AR causes immune cell activation within the nasal mucosa, along with activation of the resident epithelial cells and fibroblasts (1, 2). These cellular events induce the tissue recruitment of eosinophils. Activation of eosinophils within the epithelium underlies the development of clinical symptoms of AR through the interaction between mediators secreted by eosinophils and tissues within the nose (1, 2).

Accumulation and activation of eosinophils at the site of allergic inflammatory responses is believed to be regulated by a complex series of events that involves helper T-cells and cytokines. Cytokines, especially interleukin (IL)-3, IL5 and granulocyte-macrophage colony-stimulating factor (GM-CSF), influence eosinophil growth, maturation and migration (3, 4). In addition to these cytokines, chemokines such as regulated on activation, normal T-cell expressed and secreted (RANTES) and eotaxin, which are classified as CC chemokines, increase the ability of eosinophils to migrate from blood vessels into inflammatory sites, as well as their activation (3, 4). It is also accepted that the number of migratory eosinophils and their activation status are correlated with disease severity (5). The binding of RANTES or eotaxin to the most important CC chemokine receptor, CCR3, on eosinophils leads to several biological events, including calcium mobilization and oxygen radical production (6). This ligation also causes eosinophil degranulation, resulting in the secretion of various granule proteins such as major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase, which promote tissue remodeling after mucosal injury (7, 8). From

these points of view, it is desirable to develop anti-allergic agents that can inhibit eosinophil activation or migration by blocking eosinophil chemokine receptors or by inhibition of the production of eosinophil chemokines (6, 9).

Periostin, formerly called osteoblast-specific factor 2, was originally identified in a mouse osteoblastic cell line as a cell adhesion protein and was recently classified as a novel matricellular protein (10-13). This protein is secreted from various types of cells such as fibroblasts, epithelial cells and other structural cells after stimulation with IL4/IL13 (10, 11). Periostin has been reported to regulate fibrosis and collagen deposition (11, 14). Although periostin is known to be involved in myocardiac repair and tissue remodeling after myocardiac infarction (14), there is much evidence to indicate that periostin also plays essential roles in the development of eosinophilic airway inflammatory diseases, including AR, chronic rhinosinusitis with nasal polyps and asthma (10-12).

Quercetin is the most abundant flavonoid in human diets, and is found in foods including vegetables, fruits, tea, and wine (15). For many years, quercetin has been studied in terms of its possible health benefits, and these studies have shown that quercetin can function as a scavenger of free radicals, which damage cell membranes and even cause cell death (16). It has also been reported that quercetin can inhibit the release of histamine and other mediators, which are responsible for the development of allergic reactions, from both mast cells and eosinophils (17, 18). Furthermore, oral administration of quercetin has been reported to alleviate the ocular and nasal symptoms observed in patients with pollinosis (19). This attenuating effect of quercetin on the clinical symptoms of allergic reactions has also been observed in experimental animal models of allergic asthma (20, 21). Although these reports strongly suggest that quercetin will be a good candidate as a dietary supplement for prevention of the development of allergic diseases such as AR and asthma, the influence of quercetin on periostin functions is not fully understood. The present study, therefore, was undertaken to examine the influence of quercetin on periostin functions by using an *in vitro* cell culture technique.

Materials and Methods

Reagents. Anhydrous quercetin (CAS No. 117-39-5) was purchased from SIGMA-Aldrich Co. Ltd. (St Louis, MO, USA). According to the manufacturer's data sheet, this quercetin extracted from onions was preservative-free type, and purified with column chromatography. The purity was ≥98% as assessed by high-performance liquid chromatography. Preservative-free, pure leflunomide (CAS No. 75706-12-6) was also obtained from SIGMA-Aldrich Co. Ltd.. These two agents were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1.0 M, diluted with Airway Epithelial Cell Growth Media (AECG medium; PromoCell GmbH, Heidelberg, Germany), sterilized by passing through 0.2-μm filters and stored at 4˚C until used. Recombinant human IL4 and periostin were purchased from R & D Systems, Inc. (Minneapolis, MN, USA) as preservative-free pure powders. These proteins were dissolved in AECG medium, sterilized, and stored at 4˚C until used.

Cell culture. Human nasal epithelial cells (HNEpC), purchased from PromoCell GmbH, were suspended in AECG medium (PromoCell GmbH) at a density of 1×10^5 cells/ml and were used as a target cell. To examine the influence of IL4 on periostin production from HNEpC, 1×10^5 cells (1.0 ml) were introduced into 24-well culture plates in triplicate and were stimulated with different concentrations of IL4 in a final volume of 2.0 ml. After 24 to 96 h, culture supernatants were collected and stored at −40˚C until used. To prepare culture supernatants for examination of the influence of quercetin or leflunomide on periostin production from HNEpC after IL4 stimulation, 1×10^5 cells (1.0 ml) were introduced into each well of 24-well culture plates in triplicate that contained different concentrations of either quercetin or leflunomide. The cells were then stimulated with 15.0 ng/ml IL4 for 72 h in a total volume of 2.0 ml and culture supernatants were treated as above. To examine the influence of periostin on the production of the eosinophil chemoattractants, RANTES and eotaxin, from HNEpC, 1×10^5 cells were cultured in triplicate wells with 2.0 ng/ml periostin in the presence or absence of quercetin for 72 h. For examination of the influence of quercetin on activation of transcription factors nuclear factor-kappa B (NF-κB) and signal transducer and activator of transcription 6 (STAT6), and mRNA expression in HNEpC, 1×10^5 cells were cultured in similar manner for 24 and 48 h, respectively. In all experiments, quercetin and leflunomide were added to the cell cultures 2 h before stimulation.

Assay of factors in culture supernatants. Periostin levels in culture supernatants were examined in duplicate using commercially available enzyme-linked immunosorbent assay (ELISA) test kits (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) according to the manufacturer's recommended procedures. RANTES and eotaxin levels in culture supernatants were assessed in duplicate using ELISA test kits purchased from R & D Systems, Inc. according to the manufacturer's recommendations. The minimum levels detectable by these ELISA kits were 0.027 ng/ml for periostin, 2.0 pg/ml for RANTES and 5.0 pg/ml for eotaxin.

Assay of transcription factor activation. STAT6 activity in cultured cells was analyzed by examining the level of phosphorylated STAT6 using an ELISA test kit (R & D Systems, Inc.). NF-κB activity was measured using commercially available NF-κB p65 ELISA test kits (Active Motif Co. Ltd., Carlsbad, CA., USA). Test kits were used according to the recommended procedures.

Assay of mRNA expression. Poly A^+ mRNA was separated from cultured cells using oligo(dT)-coated magnetic micro beads (Miltenyi Biotec, Bergisch Gladbach, Germany). The first-strand cDNA was synthesized from 1.0 μg of Poly A^+ mRNA using a Superscript cDNA synthesis kit (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. A polymerase chain reaction (PCR) was then carried out using a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The PCR mixture consisted of 2.0 μl of sample cDNA

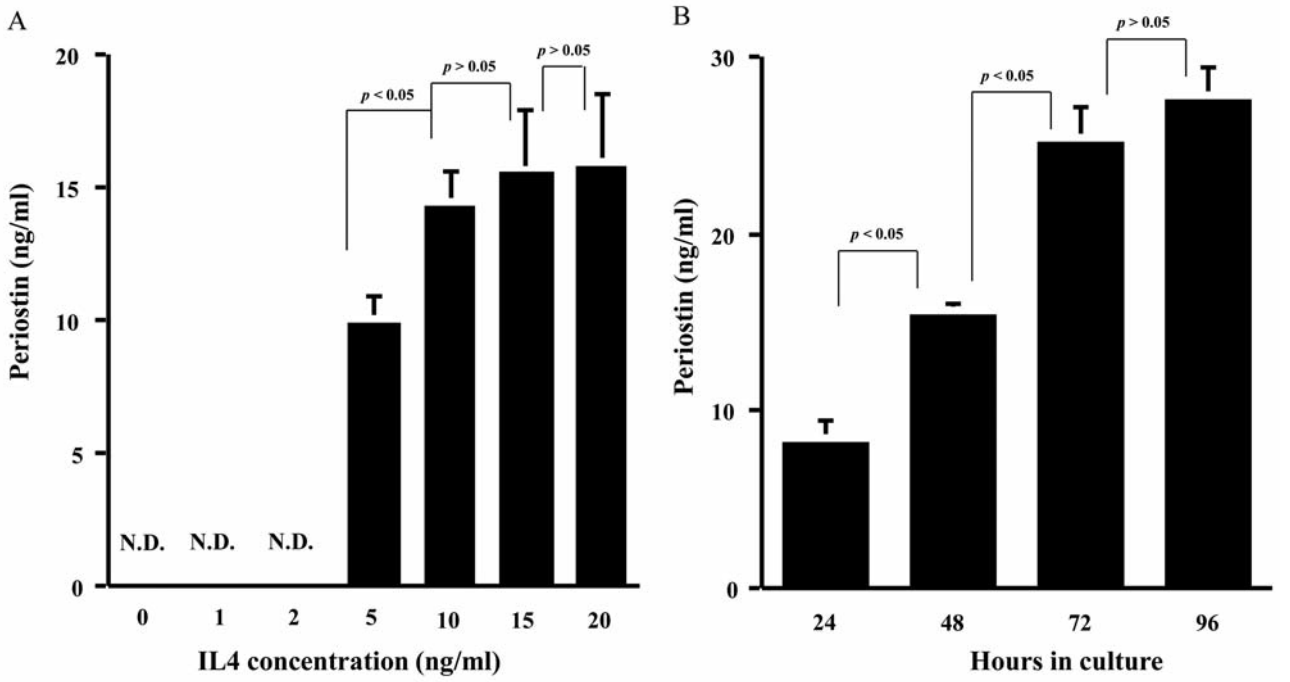

Figure 1. *Influence of interleukin (IL)-4 stimulation on periostin production by human nasal epithelial cells (HNEpC) in vitro. HNEpC at 1×10^5 cells/ml were cultured with different concentrations of IL4 for 48 h (A) or with 15 ng/ml IL4 for the indicated time (B). The concentration of periostin in the culture supernatants was then measured using enzyme-linked immunosorbent assay. The results are expressed as the mean±SE of triplicate cultures. One representative experiment of two is shown. N.D., Not detected (<0.027 ng/ml).*

solution (100 ng/μl), 25.0 μl of SYBR-Green Mastermix (Applied Biosystems), 0.3 μl of both sense and antisense primers, and distilled water to give a final volume of 50.0 μl. The reaction was conducted as follows: 4 min at 94˚C, followed by 40 cycles of 15 s at 95˚C and 60 s at 60˚C. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was amplified as an internal control. mRNA levels were calculated by using the comparative parameter threshold cycle and were normalized to that of *GAPDH*. The nucleotide sequences of the primers used were as follows: for *GAPDH*, 5'ATCTGGCACCACACCTTCTACAATGAGCTGCG-3' (sense) and 5'-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3' (antisense)11; for *RANTES*, 5'-ACC ATG AAG GTC TCC GCG-3' (sense) and 5'-TTC AGG TTC AAG GAC TCT CCA-3', and for eotaxin, 5'-AAG GCC CCT CAT TCA TCA G-3' (sense) and 5'-TTC CTT GGA AAA TGC CTT TG-3' (antisense) (9).

Statistical analysis. Statistical significance of differences between control and experimental groups was examined by ANOVA followed by Dunett's multiple comparison test. Data analysis was performed by using ANOVA for Mac (SPSS Inc., Chicago, IL, USA). The level of significance was considered at a *p*-value of less than 0.05.

Results

Influence of quercetin on periostin production by HNEpC after IL4 stimulation. The optimal concentration of IL4 required for stimulation of periostin production by HNEpC was determined in initial experiments. As shown in Figure 1A, IL4 stimulation increased periostin production from HNEpC, which was first observed at 5.0 ng/ml and plateaued out at around 15.0 to 20 ng/ml. The next set of experiments was designed to examine the time course of periostin production by HNEpC in response to IL4 stimulation. As shown in Figure 1B, periostin levels in the culture supernatants gradually increased over time, and peaked at 72 h after stimulation. The third experiment was designed to examine the influence of quercetin on the production of periostin in response to IL4 stimulation. Addition of quercetin to the cell culture caused a dose-dependent suppression of IL4-induced periostin production. The minimum concentration of quercetin that caused significant inhibition of periostin production was 4.0 μM (Figure 2). The fourth experiment was undertaken to examine the influence of the STAT6 inhibitor, leflunomide, on periostin production from HNEpC after IL4 stimulation. As shown in Figure 3, treatment of cells with leflunomide at concentrations of 100 μM or higher significantly suppressed the ability of cells to produce periostin in response to IL4 stimulation.

Influence of quercetin on STAT6 phosphorylation in HNEpC after IL4 stimulation. The fifth experiment was designed to examine the influence of quercetin on transcription factor

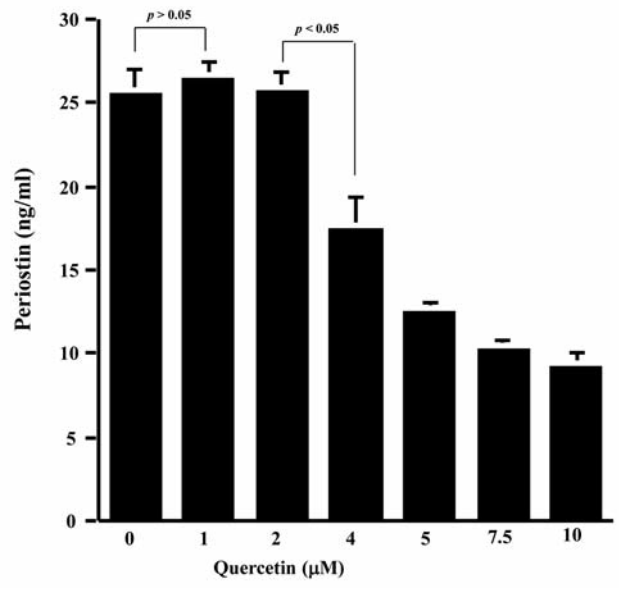

Figure 2. *Influence of quercetin on periostin secretion from human nasal epithelial cells (HNEpC) after interleukin (IL)-4 stimulation in vitro. HNEpC at 1×10^5 cells/ml were cultured with 15.0 ng/ml IL4 for 72 h in the presence of the indicated concentrations of quercetin. The periostin concentration in the culture supernatant was then measured using enzyme-linked immunosorbent assay. The results are expressed as the mean±SE of triplicate cultures. One representative experiment of two is shown.*

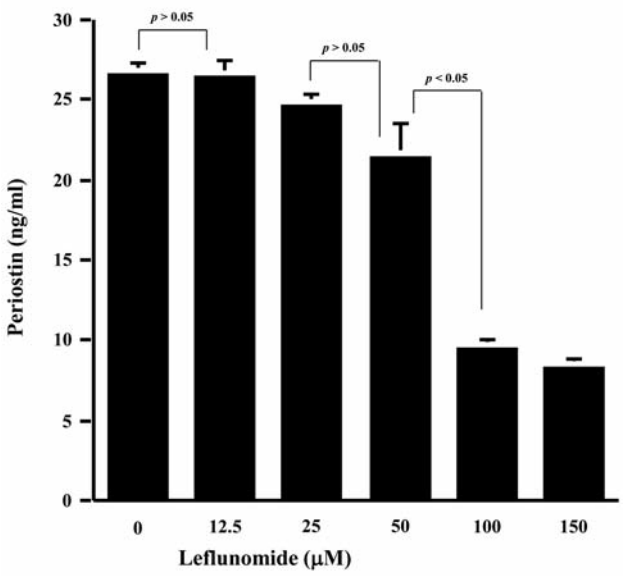

Figure 3. *Influence of signal transducer and activator of transcription 6 inhibitor leflunomide on periostin secretion from human nasal epithelial cells (HNEpC) after IL4 stimulation in vitro. HNEpC at 1×10^5 cells/ml were cultured with 15.0 ng/ml IL4 for 24 h in the presence of the indicated concentrations of leflunomide. The periostin concentration in the culture supernatant was then measured using enzyme-linked immunosorbent assay. The results are expressed as the mean±SE of triplicate cultures. One representative experiment of two is shown.*

activation in HNEpC in response to IL4 stimulation. As shown in Figure 4, addition of quercetin at concentrations of 4.0 μM or higher to cell cultures significantly suppressed STAT6 phosphorylation that was increased by IL4 stimulation.

Influence of quercetin on the production of RANTES and eotaxin by HNEpC after periostin stimulation. The sixth set of experiments was carried-out to examine the influence of quercetin on the production of RANTES and eotaxin by HNEpC after periostin stimulation. We firstly examined whether periostin stimulation could cause the production of RANTES and eotaxin by HNEpC. As shown in Figure 5, stimulation of HNEpC with periostin increased the ability of cells to produce RANTES and eotaxin in a dose-dependent manner. The minimum concentration of periostin that caused significant RANTES and eotaxin production was 2.0 ng/ml. We then examined the influence of quercetin on the production of RANTES and eotaxin by HNEpC after periostin stimulation. As shown in Figure 6A, treatment of cells with quercetin at a concentration of 4.0 μM or higher, caused significant suppression of periostin-induced RANTES production. The data in Figure 6B also show a suppressive activity of quercetin on periostin-induced eotaxin production from HNEpC, at concentrations of quercetin of 4.0 μM or higher.

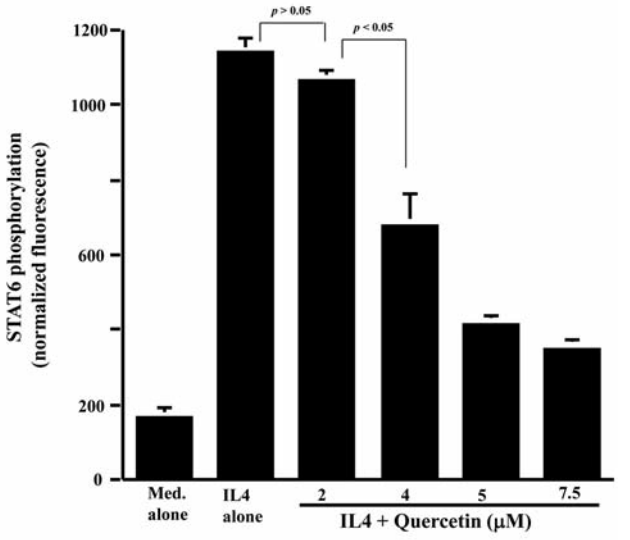

Figure 4. *Influence of quercetin on signal transducer and activator of transcription 6 (STAT6) phosphorylation in human nasal epithelial cells (HNEpC) after interleukin (IL)-4 stimulation in vitro. HNEpC at 1×10^5 cells/ml were cultured with 15.0 ng/ml IL4 for 24 h in the absence or presence of the indicated concentrations of quercetin. STAT6 activity was then measured using enzyme-linked immunosorbent assay. The results are expressed as the mean±SE normalized fluorescence of triplicate cultures. One representative experiment of two is shown. Med. alone: Medium alone.*

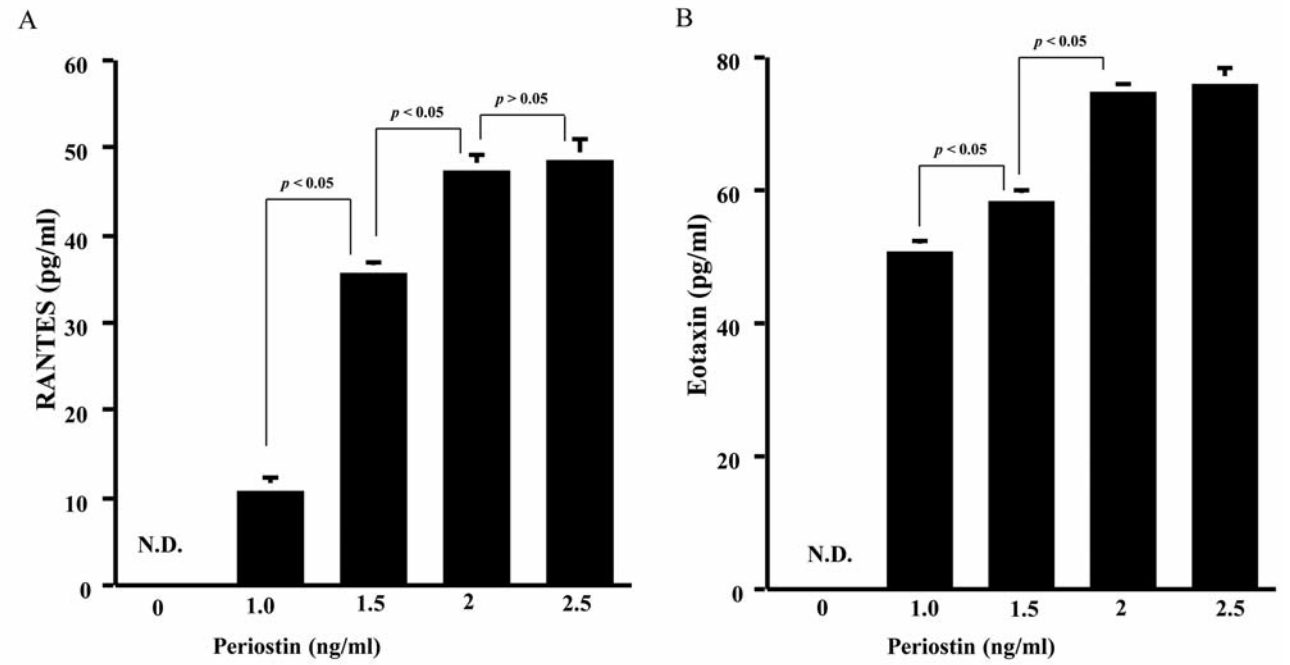

Figure 5. *Influence of periostin on the production of regulated on activation, normal T-cell expressed and secreted (RANTES) and eotaxin from human nasal epithelial cells (HNEpC) in vitro. HNEpC at of 1×10^5 cells/ml were cultured with the indicated concentrations of periostin for 72 h. The levels of RANTES (A) and eotaxin (B) in culture supernatants were then measured using enzyme-linked immunosorbent assay. The results are expressed as the mean±SE of triplicate cultures. One representative experiment of two is shown. N.D., Not detected (<2.0 pg/ml for RANTES, <5.0 pg/ml for eotaxin).*

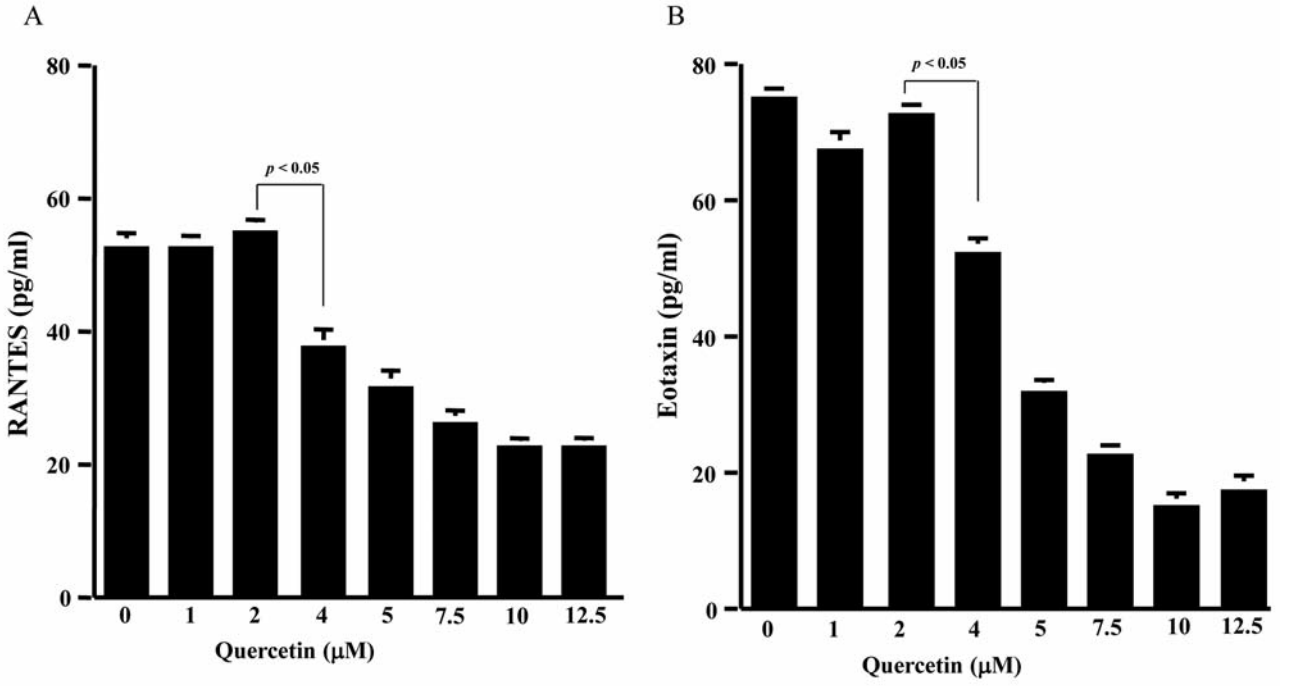

Figure 6. *Influence of quercetin on the production of regulated on activation, normal T-cell expressed and secreted (RANTES) and eotaxin from human nasal epithelial cells (HNEpC) in vitro. HNEpC at 1×10^5 cells/ml were stimulated with 2.0 ng/ml periostin in the presence of different concentrations of quercetin for 72 h. The levels of RANTES (A) and eotaxin (B) in culture supernatants were measured using ELISA. The results are expressed as the mean±SE of triplicate cultures. One representative experiment of two is shown.*

Influence of quercetin on periostin-induced NF-κB activation. The seventh experiment was carried out to examine the influence of quercetin on NFκB activation in HNEpC after periostin stimulation. As shown in Figure 7, addition of quercetin at concentrations of more than 4.0 μM to the cell cultures caused significant suppression of the NF-κB p65 activation that was increased by periostin stimulation: thus, the optical density at 450 nm, which reflects NFκB p65 activation, was significantly lower in experimental groups than that in the control.

Influence of quercetin on the mRNA expression of RANTES and eotaxin. The final set of experiments was designed to examine the influence of quercetin on the mRNA expression of *RANTES* and eotaxin in HNEpC after periostin stimulation. Treatment of cells with quercetin at a concentration of 4.0 μM or higher caused significant suppression of the mRNA expression of *RANTES* and eotaxin that had been increased by periostin stimulation (Figure 8).

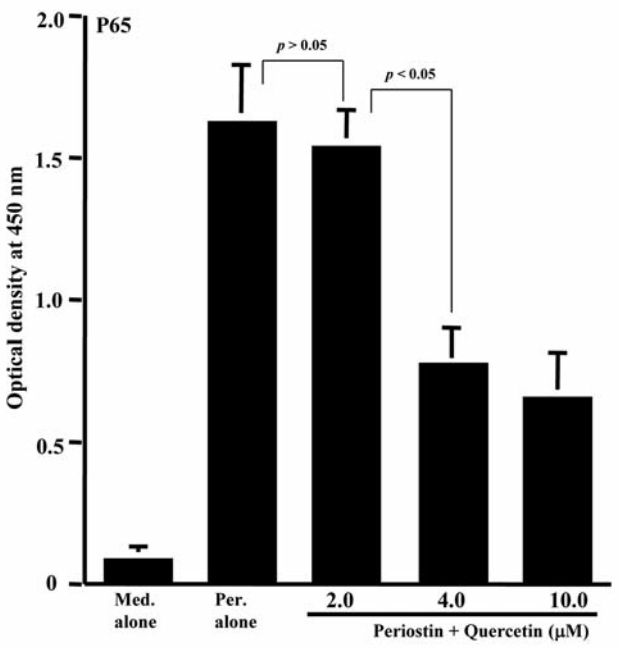

Figure 7. *Influence of quercetin on nuclear factor-kappa B (NF-κB) activity in human nasal epithelial cells (HNEpC) after periostin stimulation in vitro. HNEpC at 1×10^5 cells/ml were cultured with 2.0 ng/ml periostin in the presence of the indicated concentrations of quercetin for 24 h. NF-κB p65 activity was then measured using enzyme-linked immunosorbent assay. The results are expressed as optical density, as the mean±SE of triplicate cultures. One representative experiment of two is shown. Med. Alone, Medium alone; Per. alone, periostin alone.*

Discussion

Quercetin is the most important dietary flavonoid, and is found in high concentrations in foods such as red wine, apples and onions (15). There exists a great amount of evidence indicating that quercetin can exert anti-allergic effects through suppression of the secretion of chemical mediators such as histamines, MBP and ECP from mast cells and eosinophils *in vitro* (22-24). It has also been reported that oral administration of quercetin attenuates clinical symptoms, including bronchial hyper-reactivity to specific allergen in murine and guinea pig models of asthma (20, 21). However, the mode of action by which quercetin modulates allergic reactions is not fully understood.

It is now accepted that bodily functions are maintained in a constant, stable condition, even when the body is exposed to fluctuating conditions in the external environment. This phenomenon is called homeostasis and is regulated by the endocrine system and by endogenous peptides produced following several stimuli, as well as by the sympathetic nervous system. Out of these endogenous peptide regulators, periostin in particular has attracted attention as an important endogenous peptide in the development of allergic diseases (10-12). However, the influence of quercetin on periostin functions is not well understood. In the present study, we therefore examined the influence of quercetin on periostin functions in nasal epithelial cells *in vitro*. The present results clearly show that quercetin, at a concentration of 4.0 μM or higher, suppressed the ability of nasal epithelial cells to produce periostin in response to IL4 stimulation. Moreover, quercetin inhibited periostin-induced secretion of the eosinophil chemoattractants RANTES and eotaxin from nasal epithelial cells at similar concentrations at which it suppressed periostin production.

Periostin was originally identified in mouse osteoblasts as a cell adhesion protein and has recently been classified as a multicellular matrix protein belonging to the fascilin family (10-13). Periostin is expressed in airway epithelial cells and in lung fibroblasts, and the ability of these cells to produce periostin is increased after IL4/IL13 stimulation (10, 11). Experimental and clinical evidence clearly indicates that periostin derived from epithelial cells and fibroblasts is responsible for the development of allergic airway inflammatory diseases, including AR, as a component in sub-epithelial fibrosis (11, 12, 25, 26). Although periostin is known to play essential roles in wound healing and myocardial repair (14), its overproduction in the nasal mucosa has been reported to contribute to mucus hyper-production and edema in nasal mucosa (11), which lead to obstruction of the sinonasal passages that is observed in patients with AR (11). In an experimental mouse model of AR, compared to wild-type mice, intranasal instillation of ovalbumin into periostin-deficient mice was reported to accumulate much lower numbers of eosinophils, which are the most important final effector cells in AR, in nasal tissue (25). AR symptoms such as sneezing and nasal scratching

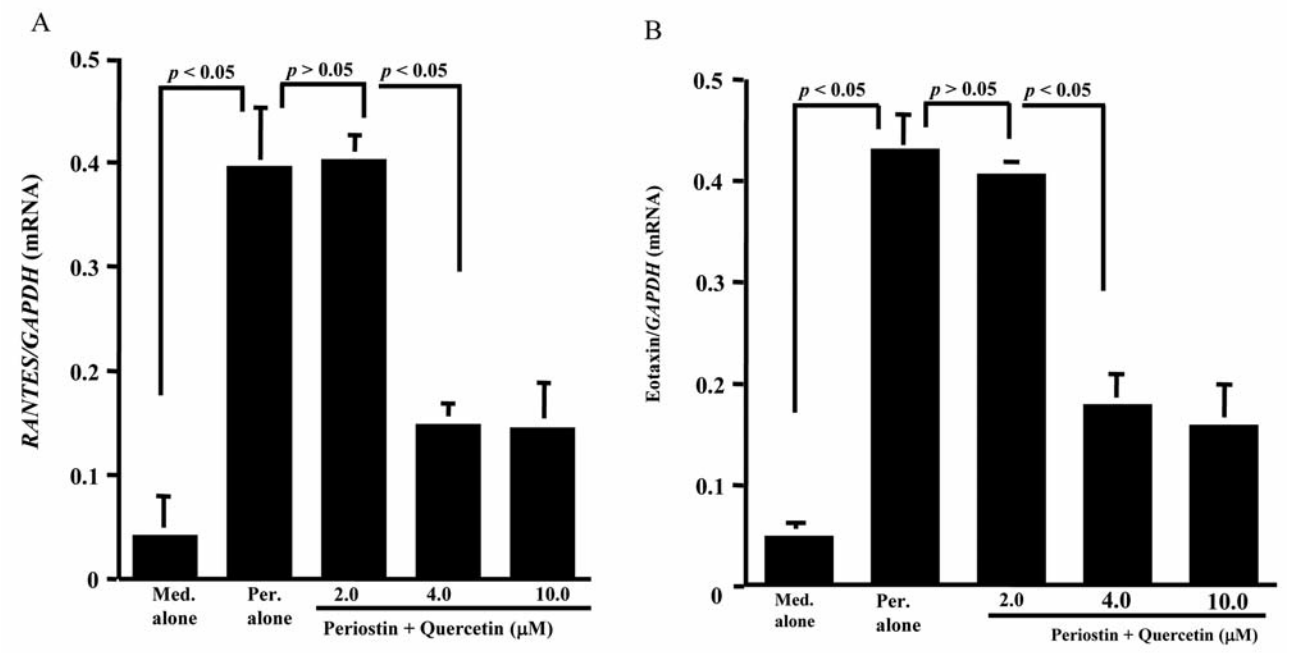

Figure 8. *Influence of quercetin on periostin-induced mRNA expression of regulated on activation, normal T-cell expressed and secreted (RANTES) and eotaxin in human nasal epithelial cells (HNEpC) in vitro. HNEpC at 1×10^5 cells/ml were cultured with 2.0 ng/ml periostin in the presence of the indicated concentrations of quercetin for 48 h. The mRNA expression of RANTES (A) and eotaxin (B) was then examined using real-time reverse transcriptase-polymerase chain reaction. The results are expressed relative to those of the target gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the mean±SE of triplicate cultures. One representative experiment of two is shown. Med. alone: Medium alone; Per. alone: periostin alone.*

motions induced by allergen challenge in periostin-deficient mice were also reported to be fewer than those observed in wild-type mice (25). After oral administration of 64 mg quercetin to humans, the plasma level of quercetin gradually increased and peaked at 650 nM (27). Commercially available preparations of quercetin generally contain 400 mg to 1,200 mg quercetin, and a daily dose of 1,200 mg to 1,500 mg quercetin is recommended as a dietary supplement (28). Assuming first-order kinetics, a 1,200 mg dose could lead to a plasma concentration of up to 12 μM (27), which is a much higher level than that at which quercetin induces suppressive effects on periostin production. Judging from these reports, the present results may suggest that oral administration of quercetin to patients with AR might cause a decrease in the ability of nasal cells to produce periostin and suppress periostin-induced inflammatory responses, resulting in favorable modification of the clinical condition of AR.

IL4 is well-established as a pleiotropic inflammatory cytokine that is mainly secreted from Th2 type T-cells and has been shown to be central to the pathogenesis of allergic disorders, including AR, atopic dermatitis and asthma (29). It is also accepted that IL4 activates several molecules that are implicated in cellular signal transduction (29, 30). IL4 first binds to the IL4 receptor alpha, leading to the activation of Janus kinases 1 and 3 (29, 30). Activation of these tyrosine kinases causes the subsequent phosphorylation and activation of STAT6, which is essential for inflammatory protein production, including of cytokines and chemokines (29, 31). Therefore, in the present study we examined whether quercetin inhibits periostin production from nasal epithelial cells after IL4 stimulation through an effect on this signaling pathway. Addition of leflunomide, a STAT6 inhibitor, at a concentration greater than 100 μM to cell cultures significantly inhibited IL4-induced periostin production, indicating that STAT6 activation is essential for periostin production from nasal epithelial cells. We also showed that quercetin inhibited STAT6 phosphorylation induced by IL4 stimulation. These results strongly suggest that quercetin inhibits periostin production from nasal epithelial cells after IL4 stimulation through suppression of STAT6 phosphorylation.

Allergic diseases such as AR and asthma are characterized by high infiltration and activation of Th2 T-cells, eosinophils, basophils and mast cells into the inflamed tissue, be it lung, nasal mucosa, or other tissue (3, 4). Eosinophils are believed to be a key player among these cells, and to contribute to the development of pathological changes in allergic diseases through secretion of harmful mediators such as MBP, ECP and free radicals (7, 8). Eosinophil trafficking to inflammatory sites from blood vessels is dependent on the coordinated actions of cytokines, chemokines and adhesion

molecules. Although IL5 is believed to be a central factor mediating eosinophil recruitment, the chemokines RANTES and eotaxin also play pivotal roles in eosinophil migration (3, 4). In addition to eosinophil-selective chemotactic activity, these two chemokines function in inflammatory cells to activate the secretion of harmful mediators, cytokines and chemokines (8). RANTES increases the ability of both mast cells and basophils to secret histamine and prostaglandins, which are important mediators in the development of inflammatory responses (9). Furthermore, eotaxin is reported to be responsible for tissue inflammation through generation of reactive oxygen species and the induction of histamine and leukotriene C4 degranulation in mast cells, basophils and eosinophils (6). RANTES and eotaxin exert their biological effects by binding to and activating the chemokine receptor, CCR3, which is expressed on both inflammatory cells and resident cells, including epithelial cells and fibroblasts (4, 6). Signals derived from the binding of chemokines and CCR3 lead to an increase in the production of matrix proteins and transforming growth factor-β, which play important roles in the development of tissue remodeling at sites of allergic inflammation (9). Combined with these reports, it is reasonable to speculate that the suppressive effects of quercetin that we observed on RANTES and eotaxin production by nasal epithelial cells in response to periostin stimulation may underlie, in part, the therapeutic mode of action of quercetin on allergic diseases, including AR.

Periostin has been shown to activate NF-κB in keratinocytes and fibroblasts, resulting in an increase in the ability of these cells to produce cytokines and chemokines (12, 32). These findings may suggest that quercetin inhibits NF-κB activation after periostin stimulation, thereby resulting in the suppression of chemokine secretion by nasal epithelial cells. This speculation may be supported by the observation that addition of quercetin to cell cultures caused inhibition of NF-κB activation induced by periostin stimulation. NF-κB activation requires an increase in Ca^{2+} level in the cytosol (33). It is reported that quercetin can inhibit the increase in intracellular Ca^{2+} level that is induced by inflammatory stimulation of human mast cells *in vitro* (34). Quercetin is also reported to inhibit the activation of several types of protein kinases that are responsible for transcription factor activation (22, 35). These reports suggest the further possibility that quercetin inhibits the activation of protein kinases through inhibition of changes in Ca^{2+} levels in nasal epithelial cells after periostin stimulation, thereby resulting in suppression of chemokine production. Further experiments are required to clarify this point.

Conflicts of Interest

The Authors declare that there exist no conflicts of interest regarding the publication of this article.

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