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The Suppressive Effect of Quercetin on Toll-Like Receptor 7-Mediated Activation in Alveolar Macrophages

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Key Words
Quercetin · Flavonoids · Heme oxygenase-1 · Toll-like receptor 7

Abstract
Respiratory viral infections that cause chronic airway and lung disease can result in the activation of the innate immune response. Alveolar macrophages (AMs), one of the first lines of defense in the lung, are abundantly located in alveoli and the respiratory tract. Flavonoids found in fruits and vegetables exhibit cytoprotective effects on various cell types. In this study, we investigated the effect of quercetin on activation of AMs that had been exposed to imiquimod, a ligand of Toll-like receptor (TLR) 7. In both a mouse AM cell line (AMJ2-C11 cells) and mouse bronchoalveolar fluid cells, we demonstrated that quercetin attenuated TLR7-induced the expression of TNF-α and IL-6. In AMJ2-C11 cells, quercetin also attenuated the TLR7-induced CD40 expression; attenuated the translocation of p65; induced translocation of Nrf2 from cytosol to nucleus; and induced heme oxygenase (HO)-1 expression. Notably, tin protoporphyrin IX (SnPP), an inhibitor of HO-1, also attenuated TLR7-induced transcription of the TNF-α and IL-6 genes, suggesting that the effect of quercetin is mediated by HO-1. These results suggest that dietary supplementation with quercetin may have efficacy in the treatment of respiratory viral infection.

Introduction

Viral respiratory tract infections cause pneumonia and exacerbations of chronic lung diseases, such as asthma [1]. The airway epithelial cells, alveolar macrophages (AMs), and dendritic cells recognize the component of an invading virus through host pattern-recognition receptors. After recognition of viral components, pattern-recognition receptors initiate an efficient antiviral response and trigger a specific adaptive immune response [1–4].

AMs are a key component of pulmonary cell-mediated immune responses in the lung [5, 6]. In the steady state, AMs prevent the development of specific immune responses by performing phagocytosis and sequestration of antigen from the immune system [3, 6, 7]. Once activated, AMs become efficient effector cells that participate in phagocytosis, killing, and coordination of the innate immune response [6]. With respect to respiratory viruses,

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some members of the Toll-like receptor (TLR) subfamily recognize viral nucleic acid. Specifically, TLR3 detects viral double-stranded RNA; TLR7 and human TLR8 identify viral single-stranded RNA; and TLR9 recognizes viral DNA [2, 8]. These TLRs are localized to intracellular vesicles, which contain targeting sequences for the induction of immune responses against viral infections [2, 8–13]. After engagement of TLRs by their cognate pathogen-associated molecular patterns, the nuclear factor-kappa B (NF-κB) is activated, resulting in the induction of genes involved in inflammatory responses [2].

Flavonoids are low molecular weight compounds found in many plants. These compounds exhibit anti-viral, antioxidant, anti-allergic, and anti-inflammatory activities [14]. Our previous studies on quercetin, one of the most common flavonoids, indicated that quercetin exerts cytoprotective effects via the heme oxygenase (HO)-1-dependent pathway in mast cells, fibroblasts, and alveolar epithelial cells [15–17]. HO-1 is a stress-inducible enzyme that exhibits cytoprotective effects against oxidative stress, a role mediated through the induction of the antioxidant activities of biliverdin and its metabolite, bilirubin, and by induction of carbon monoxide, which has anti-inflammatory effects [18]. Quercetin has been reported to counteract the activation of macrophage and dendritic cells caused by TLR-ligand interactions [19–21]. However, the possible role of quercetin in attenuating TLR7-mediated activation has not yet been elucidated. To facilitate the characterization of quercetin's effect on TLR7-associated processes, we employed imiquimod, a synthetic ligand of TLR7 that is a potent activator of anti-viral immune cells; imiquimod also directly activates macrophages to produce local cytokines such as TNF-α and IL-12 [22]. In this study, we investigated the effects of quercetin on macrophages activated by imiquimod, a model that may mimic a physiologically relevant in vivo infection by single-stranded RNA viruses. We also investigated the possible involvement of HO-1 induction by quercetin in TLR7-activated AMs.

Materials and Methods

Reagents

Quercetin was obtained from Sigma-Aldrich (St. Louis, Mo., USA). Tin protoporphyrin IX (SnPP) was purchased from Frontier Scientific (Logan, Utah, USA). Imiquimod was obtained from Calbiochem (Darmstadt, Germany).

Animals

Wild-type C57BL/6 mice were purchased from SLC (Shizuoka, Japan). Animals were maintained in a temperature (22–24°C), humidity (55 ± 5%), and light (12 h light-dark cycle; lights on at 8:00) regulated room with access to food and water ad libitum. Animal experiments were approved by the Animal Experiment Committee of Nagoya University Graduate School of Medicine. All procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagoya University.

Collection of Bronchoalveolar Lavage Fluid Cells

Bronchoalveolar lavage fluid (BALF) was collected as previously described [23]. Briefly, mice were exsanguinated by aortic perforation under pentobarbital anesthetization. The trachea was cannulated and the lungs were lavaged 6 times with PBS (0.5 ml each time). Collected BALF were centrifuged at 1,200 rpm for 3 min, and the pelleted cells were then re-suspended in RPMI-1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, MEM non-essential amino acids, 1 mmol/l sodium pyruvate, 50 μmol/l 2-mercaptoethanol, and 10% FBS. More than 95% of BALF cells were macrophages as assessed by May-Grünwald and Giemsa staining.

Cell Culture

The murine AM cell line, AMJ2-C11, and the murine monocyte/macrophage cell line, RAW264.7, were purchased from the American Type Culture Collection (Manassas, Va., USA). AMJ2-C11 cells were maintained in Dulbecco's modified Eagle's medium (Wako Pure Chemical Industries, Japan) supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, MEM non-essential amino acids, and 1 mmol/l sodium pyruvate (Wako Pure Chemical Industries). RAW264.7 cells were maintained in RPMI-1640 medium (Wako Pure Chemical Industries) containing 100 U/ml penicillin, 0.1 U/ml streptomycin, 0.25 μg/ml amphotericin B, 1 mmol/l sodium pyruvate, MEM non-essential amino acids, and 10% FBS. Cells were incubated at 37°C in a 5% CO_2 atmosphere.

Cell Viability Assay

AMJ2-C11 cells were incubated with or without quercetin. Twenty four hours after adding quercetin, a colorimetric assay was performed based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. WST-1 (10%/well) was added to each well, and cells were incubated for 1 h. The plate was read at a wavelength of 450 nm with a reference at 650 nm.

Reverse Transcription-PCR and Quantitative Real-Time PCR

Total RNA was isolated using ISOGEN II (Nippon Gene, Toyama, Japan) and reverse-transcribed to cDNA using random hexamers (Invitrogen, Carlsbad, Calif., USA) and ReverTra Ace (TOYOBO, Osaka, Japan) according to the manufacturer's instructions. Quantitative real-time PCR was performed on an ABI 7000 Sequence Detector (Applied Biosystems, Foster City, Calif., USA). Primers and probes for *Tnfa*, *Il6*, *Hmox1*, *Nqo1*, and *Gapdh* were obtained from Nippon EGT (Toyama, Japan). Relative quantities of expression were estimated by the standard curve method. The results were calculated as relative (normalized) expression levels, such that the mean value for *Tnfa*, *Il6*, *Hmox1*, or *Nqo1* was divided by the mean value of *Gapdh* (a house-keeping gene quantified as an endogenous reference RNA). Ratios were then calculated by dividing the normalized values obtained for stimulated cells by those obtained for control cells.

ELISA

BALF cells (2×10^5 cells/well) were cultured with 20 μmol/l quercetin for 1 h after the treatment of 20 μmol/l SnPP for 30 min and stimulated with 10 μmol/l imiquimod for 24 h. The supernatant was collected, and the production of TNF-α and IL-6 was assayed using a murine ELISA development kit (PEPROTECH, Roky Hill, N.J., USA). Briefly, flat-bottomed 96-well plates were coated with goat anti-murine TNF-α or rabbit anti-murine IL-6, and incubated at room temperature overnight. Plates were then washed and blocked for 1 h at room temperature with PBS containing 1% bovine serum albumin (Sigma-Aldrich). The supernatants were then added and plates were incubated for 2 h at room temperature. Subsequently, biotinylated goat anti-murine TNF-α or rabbit anti-murine IL-6 was added and plates were incubated for 2 h at room temperature. After washing, plates were incubated with avidin-horseradish peroxidase for 30 min. The production of TNF-α and IL-6 in the supernatant were assessed by a colorimetric assay using the peroxidase substrate TMB (Becton Dickinson, Franklin Lakes, N.J., USA). The absorbance was measured at 450 nm using a microplate reader.

Western Blotting

Twenty μg of protein was subjected to SDS-PAGE using 12% separation gel and transferred to polyvinylidene difluoride membrane (Millipore, Billerica, Mass., USA). The membrane was blocked by incubation (1 h at room temperature) with 2% skim milk or 2% bovine serum albumin. The membrane was then incubated (overnight at 4°C) with primary antibodies. Subsequently, the membrane was incubated (1 h at room temperature) either with anti-rabbit IgG or with anti-mouse IgG antibodies conjugated with horseradish peroxidase. The specific protein bands on the membrane were visualized using the Enhanced Chemiluminescence Kit (Millipore) according to the recommendations of the manufacturer. The intensity was quantified using Scion image software (Scion Corporation, Frederick, Md., USA).

Cell Lysate Preparation and Nuclear Fractionation

Whole-cell lysates were prepared in lysis buffer (20 mmol/l HEPES (pH 7.5), 150 mmol/l NaCl, 1 mmol/l EDTA-2Na, 1% Triton-X) containing protease inhibitor cocktail. The nuclear fractionation of whole cells was performed using DUALXtract Nuclear and Cytosolic Protein Extraction kit (Dualsystems Biotec, Schlieren, Switzerland). Briefly, cells were treated with cell lysis buffer. After centrifugation, the nuclear pellet was washed twice and lysed with nuclear lysis reagent. After centrifugation, the cleared supernatant was used as the nuclear protein extract.

Flow Cytometric Analysis of Cell-Surface Molecule Expression

After stimulation with imiquimod for 24 h, cells were incubated (20 min at room temperature) with FITC or PE-conjugated primary antibodies (anti-CD40, anti-CD80, anti-CD86, anti-CD23, and anti-MHC class II). The expression of cell-surface molecules was analyzed by a FACSCalibur flow cytometer (Becton Dickinson).

Immunofluorescent Staining

AMJ2-C11 cells were cultured in LabTek chamber slides (Thermo Scientific, N.Y., USA) and then incubated with quercetin for 1 h. Slides were fixed in Histochoice (Amresco, Solon, Ohio, USA) for 30 min and permeabilized with 1% Triton X-100 in PBS, then incubated (overnight at 4°C) with primary anti-nuclear factor E2-related factor 2 (Nrf2) polyclonal antibody. Next, slides were rinsed with PBS and incubated (1 h at room temperature) with FITC-labeled anti-rabbit IgG antibody. Nuclei were visualized by staining with propidium iodide (Vector, Burlingame, Calif., USA). Immunofluorescence was observed using an AX80 microscope (Olympus, Tokyo, Japan).

Statistical Analysis

Statistical comparisons among the groups were assessed by one-way analysis of variance. When F ratios were significant ($p < 0.05$), Scheffe's post-hoc tests were used to compare groups, with $p < 0.05$ considered statistically significant. Statistical analyses were performed with StatView (Abacus Concept, Inc., Gloucestershire, UK).

Results

Effects of Quercetin on the Cell Viability of a Mouse AM Cell Line

To determine the optimal (non-toxic) concentration of quercetin for investigation, we checked the effect of quercetin for cell viability (fig. 1a). AMJ2-C11 cells were incubated with various concentrations of quercetin (0–60 μmol/l) for 24 h and subjected to the WST-1 assay. Based on the results, we used quercetin at or below concentrations of 20 μmol/l for the following experiments.

Quercetin Modulation of Imiquimod-Induced Cytokine Expression

We next examined the effect of quercetin on imiquimod-induced cytokine expression in AMJ2-C11 cells. First, we investigated the expression of TLR7 on AMs. Whole-cell lysates were prepared and subjected to western blotting using anti-TLR7 antibody. TLR7 was constitutively expressed on AMJ2-C11 cells and the expression level was much higher than on RAW264.7 cells, a murine macrophage cell line (fig. 1b). Imiquimod significantly induced the expression of mRNAs for TNF-α and IL-6 in AMJ2-C11 cells, with transcript levels increasing by 7.0- and 61.5-fold (respectively). Quercetin significantly attenuated the imiquimod-induced expression of TNF-α at the concentration of 20 μmol/l. The expression of IL-6 was dose-dependently inhibited by quercetin (fig. 1c). Furthermore, we used western blot analysis to investigate whether the effect of quercetin was mediated by the TLR7-initiated signal transduction, a pathway known to depend on the translocation of p65, a subunit of NF-κB, from the cytosol to the nucleus. Lamin A and α-tubulin were used as markers for the nuclear and cytoplasmic fractions, respectively. When AMJ2-C11 cells were pretreated with quercetin for 1 h before exposure to imiquimod, quercetin significantly attenuated imiquimod-induced translocation of p65 into the nucleus (fig. 1d).

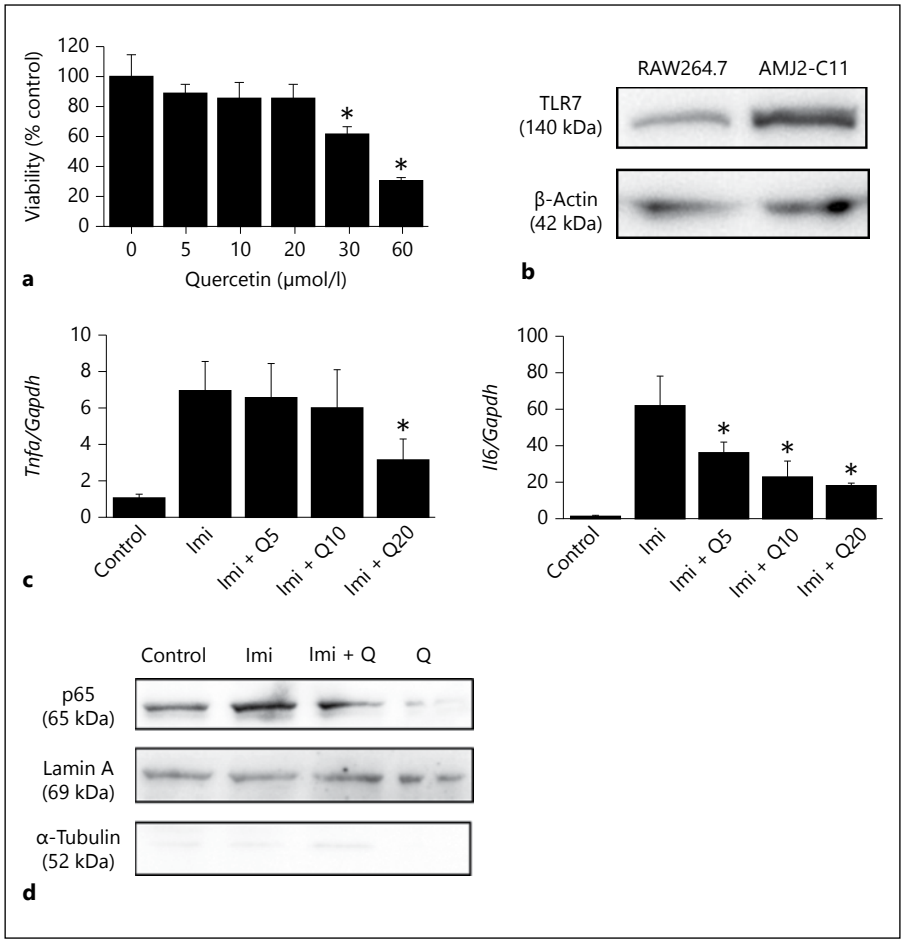

Fig. 1. Effects of quercetin on cell viability and imiquimod-induced cytokine expression. **a** AMJ2-C11 cells (1×10^4 cells/well) were exposed to various concentrations of quercetin (0, 5, 10, 20, 30, 60 μmol/l) for 24 h. At the end of the incubation, WST-1 reagent was added and cultures were incubated for another hour. Results are expressed as means ± SD of triplicate samples, and are representative of 3 independent experiments. Asterisks indicate significant differences from control cells ($p < 0.05$). **b** The expression of TLR7 in AMJ2-C11 cells and RAW264.7 cells were detected by western blotting using whole cell lysates. **c** AMJ2-C11 cells (4×10^5 cells/well) were treated with quercetin (0, 5, 10, 20 μmol/l) for 1 h and then stimulated with 10 μmol/l imiquimod for 1 h (TNF-α) or 4 h (IL-6). The level of each cytokine-encoding mRNA was determined by quantitative real-time PCR. Results are expressed as means ± SD of triplicate samples, and are representative of three independent experiments. Asterisks indicate significant differences from control cells ($p < 0.05$). **d** AMJ2-C11 cells (1×10^6 cells/dish) were treated with 20 μmol/l quercetin for 1 h and then stimulated with 10 μmol/l imiquimod for 20 min, and the expression of p65 in the nuclear fraction was determined by western blotting. Imi = Imiquimod; Q = quercetin.

Thus, quercetin's effects on cytokine expression are transduced via NF-κB activation, a step in the TLR7 signal transduction pathway.

Analysis of Cell-Surface Molecules on AMs Stimulated by Imiquimod

We next used flow cytometry to examine the expression of several cell-surface molecules, including CD40, CD80, CD86, CD23, and MHC class II. As shown in figure 2, 24 h exposure of AMJ2-C11 cells to imiquimod yielded as slight increase in the expression of CD40. Quercetin did not attenuate the expression of CD40 induced by imiquimod. CD80, CD86, CD23, and MHC class II expressions were not changed after treatment either with imiquimod or with quercetin.

Modulation of HO-1 and NQO1 Expression and Nrf2 Translocation by Quercetin

We previously reported that quercetin exerted cytoprotective effects via Nrf2-HO-1 pathway in mast cells, fibroblasts, and epithelial cells [15–17]. We next investigated whether quercetin was able to induce HO-1 in AMJ2-C11 cells. Cells were treated with quercetin (at 0–20 μmol/l) for 4 or 8 h, and the levels of HO-1 mRNA and protein were determined by quantitative real-time PCR and western blotting, respectively. At both time points, the expression of HO-1 was induced in a dose-dependent manner in AMJ2-C11 cells (fig. 3a). At the highest tested concentration of quercetin, the HO-1 transcript and protein levels increased by 2.3- and 4.1-fold (respectively) compared to those of control cultures. Since the *Hmox1* gene is regulated primarily at the transcriptional level, and its inducibility is linked to the transcription factor Nrf2 [24], we examined whether quercetin also induced another Nrf2-regulated protein, NAD(P)H:quinone oxidoreductase 1 (NQO1), in AMJ2-C11 cells. Indeed, quercetin induced the expression of NQO1 (fig. 3b). At the highest tested concentration of quercetin, NQO1 transcript and protein levels increased 4.7- and 15.9-fold (respectively) compared to those in control cul-

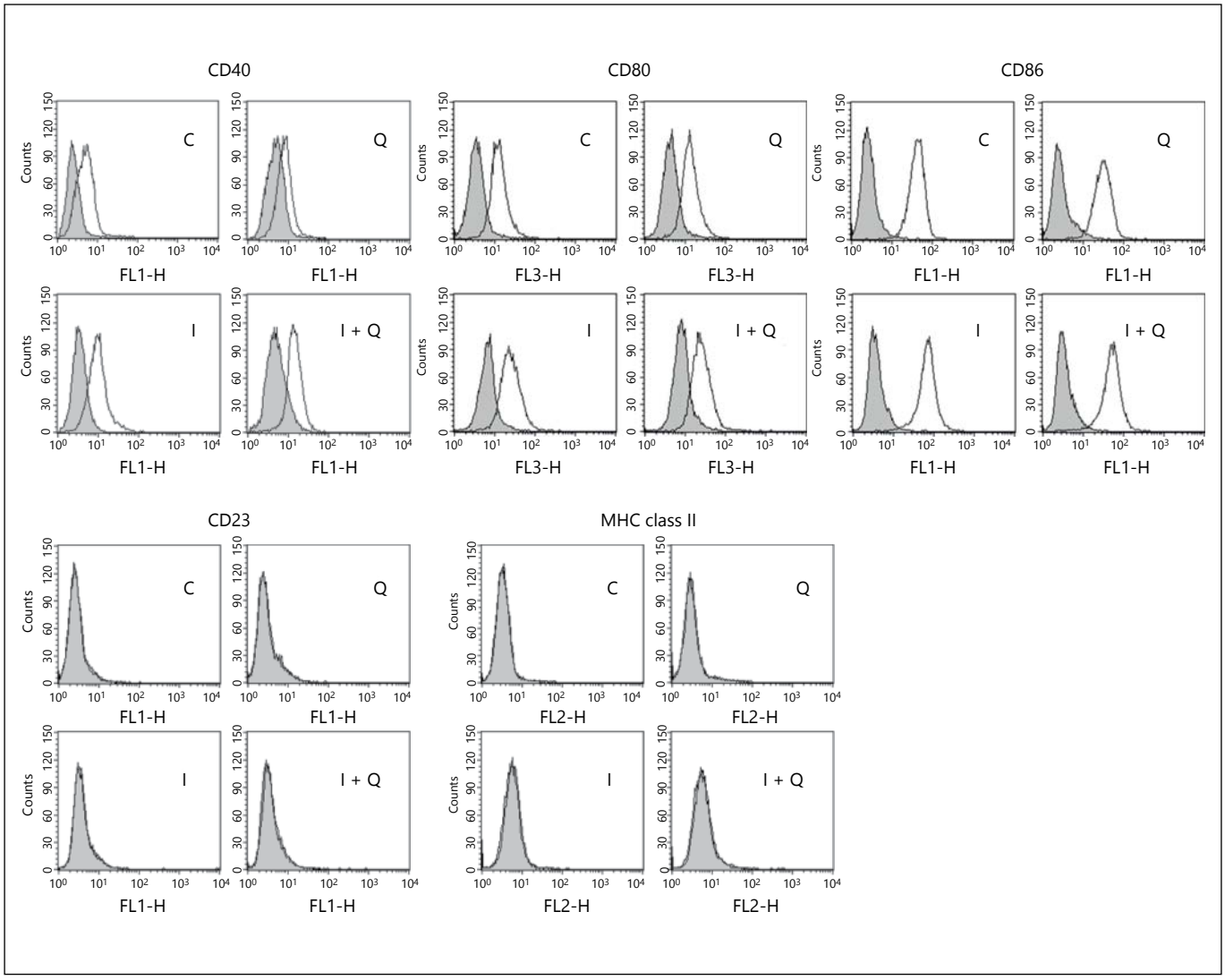

Fig. 2. Analysis of cell-surface molecules on AMs stimulated by imiquimod. AMJ2-C11 cells (1×10^6 cells/dish) were treated with 20 μmol/l quercetin for 1 h and then stimulated with 10 μmol/l imiquimod for 24 h, and the expression of CD40, CD80, CD86, CD23, and MHC class II was determined by flow cytometry. C = Control; Q = quercetin; I = imiquimod; I + Q = imiquimod + quercetin.

tures. These results suggested that the induction of HO-1 expression by quercetin was mediated by Nrf2; we therefore used western blotting to examine the localization of Nrf2 in AMJ2-C11 cells treated with quercetin (fig. 3c). Quercetin induced the translocation of Nrf2 into the nucleus after 1 h of stimulation. Cells not exposed to quercetin showed no remarkable changes in the nuclear Nrf2 levels. Immunofluorescent staining confirmed the quercetin-induced translocation of Nrf2 from the cytosol to the nucleus (fig. 3c). Together, these results suggested that the induction of HO-1 expression by quercetin in AMJ2-C11 cells was mediated by Nrf2.

Involvement of HO-1-Dependent Pathway in Quercetin Attenuation of Imiquimod-Induced Cytokine Expression

To investigate the involvement of HO-1 in the attenuation activity of quercetin, AMJ2-C11 cells were incubated with or without SnPP (20 μmol/l) for 30 min, then treated with 20 μmol/l quercetin for 1 h and stimulated with imiquimod (10 μmol/l) for 4 h. The quercetin attenuation of imiquimod-induced mRNA expression of TNF-α and IL-6 was reversed by SnPP (fig. 4), indicating that the effect of quercetin was mediated via HO-1.

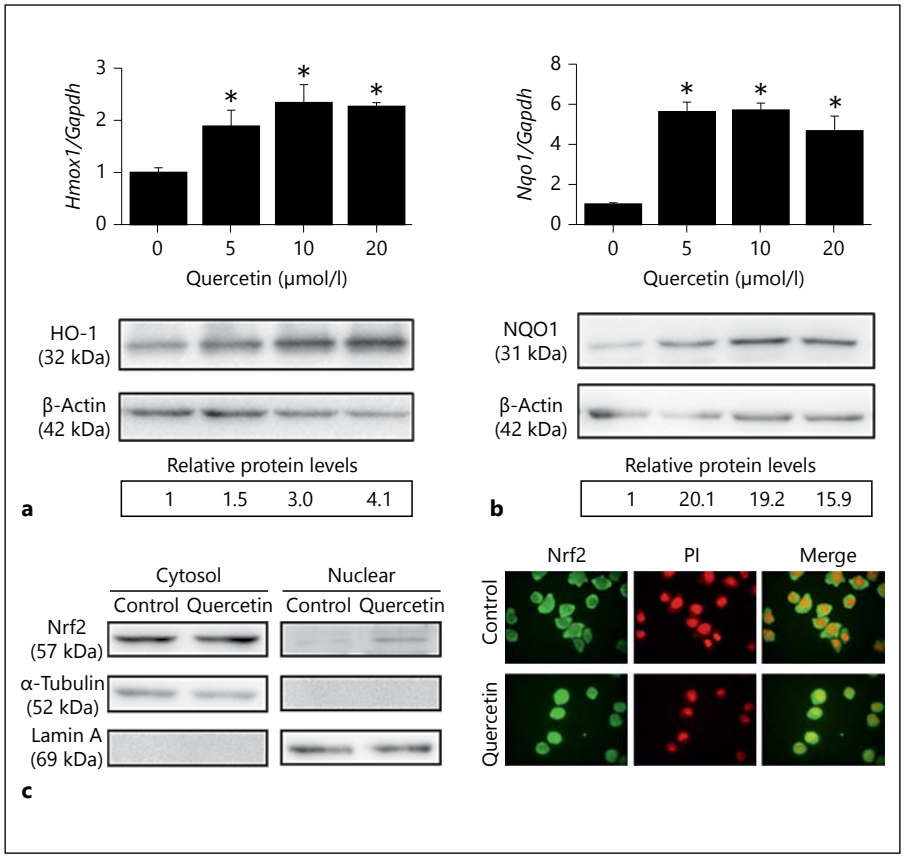

Fig. 3. Modulation of HO-1 and NQO1 expression and Nrf2 translocation by quercetin. AMJ2-C11 cells (4×10^5 cells/well) were treated for 4 h with various concentrations of quercetin (0, 5, 10, 20 μmol/l). The expression levels of mRNA and protein were determined by quantitative real-time PCR and western blotting (respectively); levels were determined for HO-1 (**a**) and NQO1 (**b**). Results are expressed as means ± SD of triplicate samples, and are representative of 3 independent experiments. Asterisks indicate significant differences from control cells ($p < 0.05$). **c** Cellular localization of Nrf2 with or without quercetin (20 μmol/l) treatment was determined by western blotting and immunofluorescent staining. Nuclear DNA was detected by propidium iodide staining. Results correspond to a single experiment that was representative of three independent experiments.

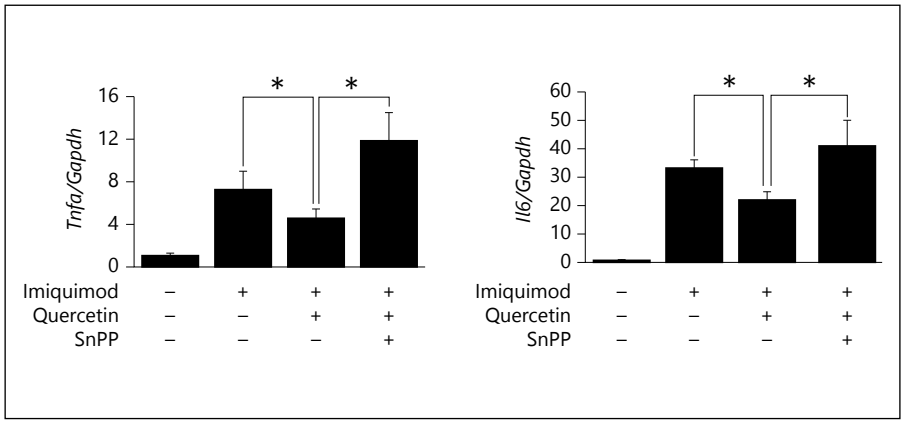

Fig. 4. Involvement of HO-1-dependent pathway in quercetin attenuation of imiquimod-induced cytokine expression. AMJ2-C11 cells (4×10^5 cells/well) were incubated with or without SnPP (20 μmol/l) for 30 min, then treated with 20 μmol/l quercetin for 1 h and stimulated with imiquimod (10 μmol/l) for 4 h. The levels of TNF-α- and IL-6-encoding transcripts were determined by quantitative real-time PCR. Results are expressed as means ± SD of triplicate samples, and are representative of 3 independent experiments. Asterisks indicate significant differences ($p < 0.05$).

Effect of Quercetin on Cytokine Production and HO-1 Expression in Mouse BALF Cells

To further investigate quercetin's activity, we examined attenuation by quercetin under ex vivo conditions. Specifically, BALF cells were isolated from C57BL/6 mice and stimulated with imiquimod after treatment with quercetin. We found that quercetin significantly inhibited imiquimod-induced TNF-α and IL-6 production, and treatment with SnPP reversed these inhibition (fig. 5a). In a separate experiment, we demonstrated that quercetin induced mRNA expression of HO-1 in BALF cells (fig. 5b). These results suggested that quercetin had cytoprotective effects ex vivo against the activation of AMs induced by imiquimod.

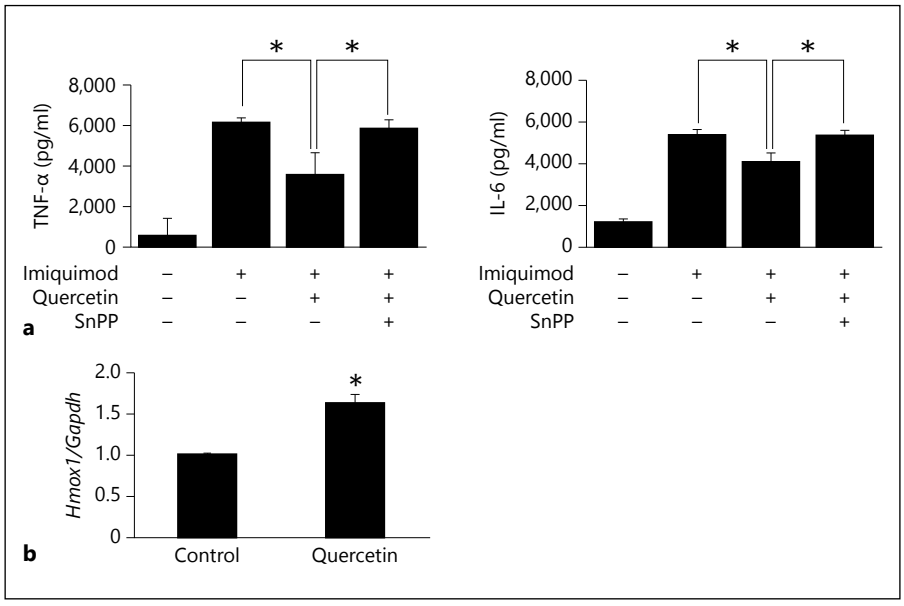

Fig. 5. Effect of quercetin on cytokine production and HO-1 expression in mouse BALF cells. **a** Mouse BALF cells (2×10^5 cells/well) were incubated with or without SnPP (20 μmol/l) for 30 min, then treated with 20 μmol/l quercetin for 1 h and stimulated with imiquimod (10 μmol/l) for 24 h. The levels of TNF-α and IL-6 were determined by ELISA. Results are expressed as means ± SD of triplicate samples, and are representative of 3 independent experiments. Asterisks indicate significant differences ($p < 0.05$). **b** Mouse BALF cells were treated with 10 μmol/l quercetin for 4 h, and *Hmox1* transcript levels were detected by quantitative real-time PCR. Asterisks indicate significant differences from control cells ($p < 0.05$).

Discussion

AMs are abundant in alveoli and the respiratory tract and have an important role in the innate immune response to infection and modulation of excessive inflammation. In this study, we investigated the effect of the flavonoid quercetin on imiquimod-induced activation of AMs. We demonstrated that quercetin exposure attenuated imiquimod-induced expression of cytokines (TNF-α and IL-6). Quercetin exposure induced the expression of HO-1 and the translocation of Nrf2 (from the cytosol to the nucleus), and the inhibitory effect of quercetin was canceled by addition of SnPP. These results suggested that quercetin-induced anti-inflammatory effect on TLR7-induced activation of AMs was mediated through the Nrf2-HO-1 pathway.

Although TLR7 and TLR8 show high sequence similarity and both recognize synthetic antiviral imidazoquinoline components [2], mouse TLR8 is not functional, even though both TLR7 and TLR8 are expressed in mice [8]. In this study, we used a mouse AM cell line and mouse BALF cells, so that imiquimod can specifically activate TLR7-mediated signaling.

TLR7 is a receptor for viral single-stranded RNA and signals through the adaptor molecule MyD88, activating NF-κB to induce proinflammatory cytokines [2]. NF-κB is generally considered to be the key transcription factor for expression of inflammatory cytokines such as TNF-α and IL-6. We showed here that quercetin decreased imiquimod-induced translocation of p65 into the nucleus in AMJ2-C11 cells. Bilirubin and biliverdin, the metabolites of HO-1, have been reported to exhibit immunomodulatory effects including the inhibition of NF-κB activity [25, 26]. We also reported that quercetin increased the levels of bilirubin in mast cells, suggesting that bilirubin and by quercetin-induced HO-1 expression reflected attenuation of NF-κB activity [15]. However, HO-1 has been reported to inhibit NF-κB activity without modulating the nuclear translocation in endothelial cells [27]. This distinction may reflect the use of different compounds and cell lines, notably, the use of deferoxamine mesylate, a chelator of Fe, to mimic the inhibitory effect of HO-1. Further studies will be needed to fully understand the inhibitory mechanism(s) of quercetin.

AMs act as professional antigen-presenting cells and are able to prime T cells through the up-regulation of the MHC class II molecules along with the co-stimulatory molecules CD40, CD80, and CD86. In this study, we showed that imiquimod slightly increased the expression of CD40, but not CD80, CD86, CD23, and MHC class II, and quercetin did not attenuate the expression levels of CD40 induced by imiquimod. These results suggested that quercetin would not affect the signaling pathway involved in CD40 expression induced by TLR7 in AMs.

Nrf2 is a transcription factor that plays an important role in a wide range of cell defense mechanisms by regulating the expression of loci encoding detoxifying enzymes such as HO-1 and NQO1 [24]. In this study, quercetin exposure induced the translocation of Nrf2 from the cytosol to the nucleus in AMJ2-C11 cells. Moreover,

quercetin induced HO-1 expression in AMJ2-C11 cells and mouse C57BL/6 BALF cells, suggesting that translocation of Nrf2 yielded increased *Hmox1* transcript levels, resulting in increased HO-1 protein expression and activity.

HO-1 is induced in response to various infections, suggesting that HO-1 is one of the host defense systems to prevent hyperactivation of the immune response [28–31]. The overexpression of HO-1 attenuates lung injury induced by the influenza virus, a single-stranded RNA virus, while the deletion of the *Hmox1* gene enhances the lung inflammation [28, 32]. In this study, we activated TLR7 signaling in AMJ2-C11 cells using imiquimod, a compound believed to mimic viral infection. Quercetin attenuated the imiquimod-induced mRNA expression of TNF-α and IL-6, and this attenuation was HO-1-dependent.

Oral supplementation of quercetin has been reported to inhibit virus replication and inflammation in lung of mice with virus infection [33, 34]. In humans, although there are few clinical trials for the inhibitory effect of quercetin in lung, Heinz et al. [35] have reported that quercetin supplementation reduced the total number of sick days and severity of upper respiratory tract infection. Since oral administration of quercetin resulted in low bioavailability by poor absorption, the plasma concentration of quercetin could be increased only up to 1.5 μmol/l even after supplementation with 1 g/day for 28 days in humans [36]. Based on these properties of quercetin, we have recently reported that direct intratracheal administration of quercetin inhibited LPS-induced lung inflammation in mice [37].

The results of our study suggested that dietary supplementation with flavonoids may serve as a therapeutic approach to preventing the development and exacerbation of viral infection-induced inflammation. Further elucidation of the mechanism of quercetin's protective effects is expected to permit the development of quercetin analogs that are more effective in HO-1 induction. Such derivatives may lead to promising new therapeutic strategies for treating a wide range of inflammatory conditions.

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Disclosure Statement

All contributing authors declare no conflicts of interest.

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