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Immunosuppressive Effect of Quercetin on Dendritic Cell Activation and Function

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Dendritic cells (DCs) play a crucial role in linking innate and adaptive immunity. Thus, DCs have been regarded as a major target of immunosuppressants for the control of harmful immune responses. In this study, we examined the effect of quercetin, a natural flavonoid found in many vegetables and fruits, on the activation and function of mouse DCs. Quercetin effectively inhibited LPS-induced DC activation by reducing the production of proinflammatory cytokines/chemokines and the expression levels of MHC class II and costimulatory molecules. In addition, quercetin uniquely blocked endocytosis by DCs and the LPS-induced DC migration was diminished by quercetin treatment. Furthermore, quercetin abrogated the ability of LPS-stimulated DCs to induce Ag-specific T cell activation, both in vitro and in vivo. Remarkably, coadministration of quercetin with 2,4-dinitro-1-fluorobenzene prevented 2,4-dinitro-1-fluorobenzene–induced contact hypersensitivity, indicating the potential of quercetin for treating delayed-type hypersensitive diseases. Blockage of LPS-induced ERK, JNK, Akt, and NF-κB activation contributed to the inhibitory effect of quercetin on DCs. These results strongly suggest that quercetin may be a potent immunosuppressive agent and could be used in the prevention and therapy of chronic inflammation, autoimmunity, and transplantation via the abolishment of DC activation and function. The Journal of Immunology, 2010, 184: 6815–6821.

Traditionally, natural products represent a source for discovering bioactive drugs, including immunomodulators, in the pharmaceutical industry (7). Flavonoids are natural products from many vegetables, fruits, herbs, flowers, seeds, and beverages that are present in diet (8). They are polyphenolic compounds and display a variety of biological effects, such as antioxidation, anti-inflammation, anticancer, gastroprotection, cardiovascular protection, oral protection, and memory improvement (9–13). In addition, flavonoid compounds have been shown to regulate immune responses (14). Thus, flavonoids may have the potential to modulate DC function in the immune system.

There are several subclasses in the flavonoid family. Quercetin (3,5,7,3′,4′-pentahydroxyflavone), which is a typical member in the flavonol subclass, is one of the most common flavonols in the diet. Its wide range of biological activities has been discussed for several decades (15). In addition, quercetin exerts antimicrobial, antihypertensive, neuroprotective, and chemoprotective effects (16). The immunomodulatory activity of quercetin has been investigated in NK cells (17), macrophages (18), mast cells (19), neutrophils (20), B cells (21), and T cells (22). Although a number of studies have reported the activity of flavonoids in the modulation of DC activation (23–27), the effect of quercetin on DCs remains unknown. In this study, we examined the immunomodulatory effect of quercetin on DC activation. Our results showed that quercetin inhibited DC maturation and function, suggesting that quercetin may be a potent immunosuppressant and may have therapeutic applications in inflammatory diseases, such as periodontitis.

Materials and Methods

Mice and generation of DCs

C57BL/6 mice were obtained from the National Laboratory Animal Center (Taipei, Taiwan). DCs were generated from mouse bone marrow, as described previously (28). OT-II TCR transgenic mice were provided by Dr. C. Lowell (University of California, San Francisco, CA). All animals were kept in a specific pathogen-free facility (National Health Research Institutes, Miaoli, Taiwan) and handled according to protocols approved by the Institutional Animal Care and Use Committee of the National Health Research Institutes.
Quercetin cytotoxicity assay

DCs were treated with quercetin (dissolved in DMSO) and made a stock solution at 25 mM (Sigma-Aldrich, St. Louis, MO), or DNFB plus quercetin (50 μM) for 16 h. Quercetin was also added to cultures 1 h before or after quercetin. The cytotoxicity of quercetin in DCs was measured using ELISA (eBioscience). All data are representative of three independent experiments.

Measurement of cytokine and chemokine production

Supernatants were collected from DCs (1 × 10^6/ml) treated with the indicated conditions. TLR ligands, including LPS (100 ng/ml), zymosan (20 μg/ml), lipoteichoic acid (20 μg/ml) (Sigma-Aldrich), polyinosine polycytidylic acid poly(1:1C) (250 μg/ml), flagellin (500 ng/ml), synthetic diacylated lipoprotein Pam2Cys-Ser-Lys4 (20 ng/ml), synthetic CpG oligodeoxynucleotides 1826 (200 ng/ml), and peptidoglycan from Staphylococcus aureus (1 ng/ml) (InvivoGen, San Diego, CA) were used to stimulate DCs. The production of cytokines (TNF-α, IL-1α, IL-1β, IL-6, IL-10, and IL-12 p70) and chemokines (MCP-1, MIP-1α, MIP-1β, and RANTES) was measured using ELISA (R&D Systems, Minneapolis, MN; PeproTech, Rocky Hill, NJ; and eBioscience, San Diego, CA).

Analysis of DC maturation and endocytosis

DC maturation was determined by the upregulation of MHC class II and costimulatory molecule expression, as described previously (29). Cells were treated with LPS (100 ng/ml), quercetin (50 μM), LPS plus DMSO (0.2%), or LPS plus quercetin for 16 h and stained with mAbs specific for mouse CD11c, I-Ab, CD40, CD80, and CD86 (BioLegend, San Diego, CA) and then analyzed by flow cytometry. The endocytosis by DCs was assessed by dextran-FITC uptake, as performed previously (30). Cells were untreated or treated with LPS (for 1 or 16 h) or quercetin (for 1 h) and then incubated with dextran-FITC. The uptake of dextran-FITC by DCs was detected by flow cytometry.

Assay of DC migration

DCs were treated with LPS (100 ng/ml), quercetin (50 μM), LPS plus DMSO (0.2%), or LPS plus quercetin for 16 h. DC chemotaxis was performed in 24-well Transwell chambers (pore size, 5 μm; Costar Corning, Lowell, MA), as described previously (31). Cells were loaded onto the upper chambers and CCL21 (200 ng/ml; PeproTech) was added to the lower chambers. After incubation for 3 h at 37°C, migrated cells were collected from the lower wells, and the number of CD11c^+ cells was determined by flow cytometry. For DC migration in vivo, cells (2 × 10^6) were labeled with CFSE for 10 min and then injected into the footpad. After 2 d, inguinal lymph nodes (LN) cells were isolated and the number of CFSE^+ cells was calculated by hemocytometry and flow cytometry.

Assay of T cell activation

Ag presentation by DCs was analyzed using the OVA-specific T cell proliferation in vitro assay, as described previously (30). Purified DCs were pulsed with OVA223–239 peptide (2 μg/ml) and incubated with LPS (100 ng/ml), quercetin (50 μM), LPS plus DMSO (0.2%), or LPS plus quercetin for 16 h. Cells were then washed with medium to remove quercetin. OT-II T cells were added to DC cultures at various DC:T cell ratios, as indicated, and T cell proliferation was determined by [3H]thymidine incorporation. For recall assay in vivo, C57BL/6 mice were immunized with OVA (10 μg; Sigma-Aldrich) and IFA (Sigma-Aldrich) mixed with quercetin (50 μg), LPS (10 μg), LPS plus DMSO, or LPS plus quercetin, via footpad injection. After 10 d, the draining LN cells were cultured with OVA at the indicated concentrations and T cell proliferation was determined by [3H]thymidine incorporation. To measure IFN-γ production, supernatants were collected from DC/OV ATII T cell and LN cell cultures, and the amount of IFN-γ was determined by ELISA (eBioscience).

Contact hypersensitivity assay

2,4-Dinitro-1-fluorobenzene (DNFB; Sigma-Aldrich)-induced contact hypersensitivity (CHS) was described previously (32). The abdomen of mice were painted with vehicle (olive oil/acetone = 1/4), DNFB (0.5% w/v), DNFB plus DMSO, or DNFB plus quercetin (50 μg) for sensitization. In addition to painting, we also used DNFB-pulsed DCs to sensitize mice. Brieﬂy, DCs were treated with vehicle, 0.1% DNFB, DNFB plus DMSO (0.2%), or DNFB plus quercetin (50 μg) for 30 min at 37°C, and 5 × 10^6 cells were then s.c. injected into mice. After 5 d, all mice were painted on the ears with DNFB (0.2%). CHS response was determined 24 h later by histological analysis using H&E staining. The increase of ear thickness was calculated as follows: 100 × (thickness of the challenged ear – thickness of the unchallenged ear)/thickness of unchallenged ear.

Western blotting

As previously described (30), DCs were treated with DMSO (0.2%) or quercetin (50 μM) and immediately stimulated with LPS (200 ng/ml). Cells were harvested and lysed at the indicated time points, and SDS-
affected the production of TNF-α in LPS-stimulated DCs, which is a hallmark of DC activation. Significantly, TNF-α was downregulated by quercetin in a dose-dependent manner (Fig. 1A), indicating that quercetin may inhibit DC activation. DMSO, the solvent of quercetin, had no effect on LPS-stimulated DCs. Next, we determined whether the suppressive effect of quercetin was caused by its cytotoxicity in DCs. Quercetin induced significant DC apoptosis at a concentration \( \geq 100 \mu M \) (Fig. 1B), which confirmed the intrinsic inhibitory effect of quercetin at concentrations \( \leq 50 \mu M \). We then examined the efficiency of the inhibitory effect of quercetin. Quercetin blocked the DC activation by LPS at high doses (up to 1 \( \mu g/ml \)) (Fig. 1C). In addition, the production of TNF-α was decreased in all tests, regardless of whether the quercetin treatment was administered to DCs before or after LPS stimulation (Fig. 1D). Furthermore, we stimulated DCs with various TLR ligands, including zymosan, synthetic diacylated lipoprotein Pam2Cys-Ser-Lys4, lipoteichoic acid, peptidoglycan (TLR1, 2, or 6), polyinosine polycytidylic acid (TLR3), flagellin (TLR5), and synthetic CpG oligodeoxynucleotides (TLR9) and found that quercetin significantly reduced TNF-α production in all conditions, although the degree of the effect varied (Fig. 1E). Collectively, we identified the inhibitory effect of quercetin on DC activation and suggested that quercetin may be a very efficient inhibitor of DC activation.

**Quercetin impaired the cytokines and chemokines produced by LPS-stimulated DCs**

In addition to TNF-α, activated DCs secrete a variety of cytokines and chemokines that regulate immune responses. Therefore, we determined whether the inhibitory effect of quercetin on TNF-α production could be extended to other cytokines and chemokines produced by LPS-stimulated DCs. As expected, the generation of cytokines (IL-1α, IL-1β, IL-6, IL-10, and IL-12 p70) (Fig. 2A) and chemokines (MCP-1, MIP-1α, MIP-1β, and RANTES) (Fig. 2B) by activated DCs was impaired by quercetin treatment. Because IL-10 production was also reduced, it is not likely that the suppressive effect of quercetin was mediated through the production of this anti-inflammatory cytokine. Importantly, the expression level of β-actin was not significantly affected by quercetin (Fig. 2C), indicating that quercetin does not have global inhibitory effect on protein expression. These results indicate that quercetin downregulates the cytokines and chemokines secreted by activated DCs, thus disturbing the immunoregulatory function of DCs.

**LPS-induced DC maturation was attenuated by quercetin**

Maturation is the key step in the DC-mediated regulation of immune responses. To investigate the effect of quercetin on DC maturation, we examined the expression levels of MHC class II and costimulatory molecules in DCs, which are the major phenotypes of DC maturation, using flow cytometry. LPS stimulation enhanced the expression of I-A^B and the costimulatory molecules CD40, CD80, and CD86 in DCs, but quercetin treatment significantly lowered the expression levels of these molecules (Fig. 3). These data suggest that quercetin attenuates LPS-induced DC maturation and limits the immunostimulatory activity of DCs.
Quercetin blocked the endocytosis by DCs

Endocytosis is a major pathway to mediate Ag uptake by DCs. Therefore, we determined the effect of quercetin on endocytosis by DCs by dextran-FITC uptake. Obviously, the ingestion of dextran-FITC was reduced in DCs after quercetin, but not LPS, treatment for 1 h (Fig. 4A). Low uptake of dextran-FITC was observed in mature DC induced by LPS stimulation for 16 h (Fig. 4B). DCs cannot become mature by stimulation for 1 h (data not shown). Thus, the blockage of endocytosis by quercetin is not related to the maturation of DCs. Our data indicate that quercetin can downregulate the endocytosis by DCs, thus impairing the Ag loading in DCs.

Migration of LPS-stimulated DCs was diminished by quercetin

DC migration is critical for the initiation of adaptive immune responses. As mature DCs are attracted by CCL19 and CCL21, we evaluated DC migration using chemotaxis assay in Transwell chambers. LPS-stimulated DCs efficiently migrated from the upper to the lower wells in response to CCL21; however, this movement was halted by quercetin (Fig. 5A). Furthermore, the migration of LPS-stimulated DCs from the footpad to the inguinal LNs was diminished by quercetin (Fig. 5B). Thus, we concluded that quercetin suppresses DC migration and disconnects the induction of adaptive immune responses.

Quercetin abrogated the Ag-specific T cell activation induced by LPS-stimulated DCs

The primary function of mature DCs is the induction of Ag-specific T cell activation. Therefore, we determined whether quercetin affected the ability of DCs to activate Ag-specific T cell activation. The abrogation of Ag-specific T cell activation induced by LPS-stimulated DCs from the footpad to the inguinal LNs was diminished by quercetin (Fig. 5A). Furthermore, the migration of LPS-stimulated DCs to the lower wells in response to CCL21; however, this movement was halted by quercetin (Fig. 5A). Therefore, we determined whether quercetin affected the ability of DCs to activate Ag-specific T cell activation. The abrogation of Ag-specific T cell activation induced by LPS-stimulated DCs from the footpad to the inguinal LNs was diminished by quercetin (Fig. 5B). Thus, we concluded that quercetin suppresses DC migration and disconnects the induction of adaptive immune responses.

CHS responses were weakened in mice cosensitized with quercetin

We identified a suppressive effect of quercetin on DCs, which implies that quercetin may prevent DC-mediated diseases. Therefore, we performed DNFB-induced CHS as a model to test this hypothesis. Mice were sensitized with DNFB in the absence or presence of quercetin via injection of DNFB-pulsed DCs (Fig. 7A) or by painting it directly onto the abdomen of animals (Fig. 7B). The CHS response to DNFB was then examined. The ears were obviously swollen in DNFB-sensitized but not in DNFB plus quercetin-sensitized mice, whereas DMSO had no effect on DNFB-sensitized mice (data not shown), indicating that quercetin inhibits the DC-mediated sensitization in CHS. These evidences suggest that quercetin has the potential to prevent delayed-type hypersensitive diseases, such as allergic contact dermatitis.
FIGURE 6. Quercetin abrogated the Ag-specific T cell activation induced by LPS-stimulated DCs. A, OT-II CD4+ T cells were cocultured with medium-, quercetin (50 μM), LPS (100 ng/ml), LPS + 0.2% DMSO-, or LPS + quercetin-treated DCs pulsed with OVA323–339 peptide (2 μg/ml) at the indicated ratios of DC:T cell. T cell proliferation was determined by [3H]thymidine incorporation after 3 d. B, C57BL/6 mice were immunized with OVA (10 μg) mixed with IFA + 0.4% DMSO, IFA + LPS (10 μg), IFA + LPS + DMSO, and IFA + LPS + quercetin (50 μg), via footpad injection. After 10 d, the draining LN cells were incubated with OVA at the indicated concentrations. T cell proliferation was measured by [3H]thymidine incorporation after 3 d. C, Supernatants were collected from cultures in A and B after 4 d. IFN-γ production was assayed by ELISA. Data shown were means ± SD of samples from three wells. NS, p > 0.05; *p < 0.05; **p < 0.01 (Student t test) was for the comparison between quercetin-treated and -untreated LPS-activated DCs. All data are representative of two to four independent experiments.

FIGURE 7. The CHS response was weakened in mice treated with quercetin. DNFB-induced CHS was described in Materials and Methods. Mice were sensitized with vehicle (olive oil/acetone = 1:4), DNFB, or DNFB + quercetin via injection of DCs (A) or by painting the abdomen of the animals (B). After 5 d, the ears of all mice were painted with DNFB and analyzed after 24 h. CHS response was determined by histology using H&E staining, and the thickness of the challenged ear was measured (original magnification ×40). Increases of ear thickness were calculated. Data shown were means ± SD of samples from six mice. ***p < 0.01 (Student t test) was for the comparison between quercetin-treated and -untreated DNFB-sensitized mice.

FIGURE 8. The activation of ERK, JNK, Akt, and NF-κB in LPS-stimulated DCs was reduced by quercetin. DCs were treated with LPS (200 ng/ml) or quercetin (50 μM) + LPS and then lysed at the indicated time points. Samples were separated on SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and then analyzed by Western blotting. The ERK, JNK, p38 MAPK, and Akt proteins (with and without phosphorylation) were detected by anti–phospho-specific and anti-protein Abs, respectively. The activation of NF-κB was determined by detecting the degradation of IκB using a specific Ab. All data are representative of three independent experiments.

In addition, the IκB degradation was decreased, indicating that the activity of NF-κB was reduced by quercetin (Fig. 8). These results suggest that quercetin suppresses DC activation via disruption of the ERK, JNK, Akt, and NF-κB pathways, which explains the strong inhibitory effect of quercetin on DC activation (Fig. 1).

Discussion

In this study, we reported for the first time that quercetin is an immunosuppressor of DCs and provided strong evidence that quercetin may be a promising agent for the prevention and treatment of inflammatory and autoimmune diseases. Our study also supports the contention that vegetables and fruits containing quercetin can promote health by reducing harmful immunity.

Quercetin is the major flavonoid and forms the backbone of many other flavonoids. The bioactivity of quercetin is probably underestimated, although it has been shown in past decades to have a large spectrum of biological effects (16). Thus, we showed that the suppression of DC function is a new activity of quercetin, and the inhibitory effect of quercetin on DCs exhibits several unique features when compared with other flavonoids. Especially, we found that the ability of DCs to take up dextran-FITC was impaired by quercetin, which represents a maturation-independent reduction of endocytosis (Fig. 4). Therefore, we reported that quercetin is the first flavonoid to inhibit endocytosis by DCs. It could be due to that quercetin binds to actin directly and then blocks the cytoskeleton rearrangement during endocytosis (33). However, other mechanisms underlying this inhibition of endocytosis by quercetin warrants further investigation.

Another unique activity is the inhibition of DC migration by quercetin. CCR7 expression has been suggested to play a major role...
in regulating the DC migration (34). However, quercetin had no effect on the expression of CCR7 in DCs after LPS stimulation (Supplemental Fig. 1), indicating the involvement of other mechanisms for the inhibitory effect of quercetin on DC migration. It is possible that the effects of quercetin on cytoskeleton rearrangement, as suggested in the inhibition of endocytosis, may also reduce DC migration (33).

Quercetin targets multiple intracellular signaling pathways in cells, such as NF-κB (35). The reduction of IκB degradation and blockade of Akt activity by quercetin in LPS-stimulated DCs (Fig. 8) are consistent with what was observed in macrophages (36, 37). However, quercetin inhibited the phosphorylation of ERK and JNK, but not p38 MAPK, in DCs (Fig. 8), which is in contrast with previous studies (38, 39). In addition to the signaling pathways examined in this study, inhibition of STAT1 activation (40), reduction of TBK1 kinase activity in the TIR domain-containing adaptor protein- inducing IFN-β–dependent signaling pathway (41), and disruption of the accumulation of lipid rafts (37) are involved in the inhibition of macrophage activation by quercetin. These pathways are probably related to the suppressive effect of quercetin on DCs.

A possible mechanism by which quercetin develops an immunosuppressive activity is the activation of the aryl hydrocarbon receptor (AhR), which is a cytosolic transcription factor that mediates the immunotoxicity of dioxin (42). AhR has recently been suggested to involve in immunoregulation and Th17 differentiation (43, 44). AhR activation induces regulatory T cell differentiation (45) and forms a complex with Stat1 and NF-κB in macrophages (46) and then modulates inflammatory responses. Recent reports suggest that AhR plays an essential role in the anti-inflammatory effect of other compounds (46, 47). Because quercetin is a ligand of AhR and DCs express high levels of AhR (48), it is likely that AhR may mediate the suppressive effect of quercetin on DCs. In addition, the fact that AhR signaling pathways downmodulate cytokine and growth factor production in other cell types would explain the ability of quercetin to reduce cytokine release in DCs (49). Although AhR is involved in development of immune system (50), DC differentiation was not affected by quercetin treatment (Supplemental Fig. 2). Collectively, quercetin may represent a novel approach to elucidate the role of AhR in DC biology.

Evidence for the possible clinical applications of quercetin is increasing. For instance, quercetin ameliorates EAE by blocking IL-12 signaling and Th1 differentiation (51), it improves the pathogenesis of a rheumatoid arthritis model after the onset of arthritis (52), and it attenuates the lethal systemic inflammation caused by endotoxemia (53). In this paper, we present an additional mechanism for the inhibitory effect of quercetin on DC migration (54) and prevents diabetes in NOD mice (55), which is a pathway that could be presumably induced by quercetin. Currently, we are testing the protective effect of quercetin on chronic marginal periodontitis, as implied by Petti and Scully (11). Because quercetin is a safe agent in vivo (56), additional clinical trials are warranted to support the suitability of quercetin for disease control.

In summary, our study demonstrates that quercetin can suppress DC activation and thus may potentially be used for the prevention and treatment of inflammation, autoimmunity, and transplantation. Our findings provide new insights into the immunopharmacology of quercetin. Recent studies showed that the bioactivity of quercetin can be enhanced by nanotechnological encapsulation (57) and that liposomes can stably and efficiently deliver Ag and quercetin for the Ag-specific suppression of inflammatory arthritis (58). Consequently, these techniques will hasten the development of the clinical applications of quercetin.
**Supplementary Figure 1.** Quercetin had no effect on the expression of CCR7 in LPS-stimulated DCs. DCs were treated with medium, quercetin (50 μM), LPS (100 ng/ml), LPS + DMSO (0.2%, v/v), or LPS + quercetin for 16 h. The expression of CCR7 was determined by real time PCR. Total RNA was isolated from DCs using TriZol reagent (Invitrogen). The cDNA was synthesized from total RNA with RevertAid First Strand cDNA Synthesis Kit (Fermentas). The mRNA expression levels were quantified by real time PCR using Light Cycler machine (Roche) with the SYBR Green PCR Master Mix (Fermentas) according to the manufacturer's instructions and normalized to the levels of β-actin mRNA. The expression of IL-12p40 was as a control for the inhibitory effect of quercetin. The expression level of mRNAs from untreated DCs represented as one fold. The sequences of primers used were listed as following: CCR7, F: 5’-AGAGGCTCAAGACCATGACGGA-3’ and R: 5’-TCCAGGACTTGGCTTCGCTGTA-3’; IL-12p40, F: 5’-GGAAGGCAGGCAGCAGAATA-3’ and R: 5’-AACCTTGGAGGAGAATTAGGATGG-3’ (where F= forward and R= reverse). NS, p>0.05; **, p<0.01 (Student’s t-test).
Supplementary Figure 2. Quercetin did not affect the DC differentiation. (A) Bone marrow cells were cultured in RPMI-1640 medium plus 10 ng/mL GM-CSF without or with DMSO (0.05% v/v) or quercetin (12.5 μM). Fresh media without or with DMSO or quercetin were supplied on day 3 and 5. The percentage of CD11c+ cells was determined by flow cytometry on day 3, 5, and 7. (B) GM-CSF- and Flt3L-expressing B16 melanoma cells were subcutaneously injected into B6 mice. Quercetin (15 mg/kg) or the same volume of DMSO was treated i.p. every two day. After 10 days, splenocytes were isolated and the percentage of CD11c+ cells was determined by flow cytometry. NS, p>0.05 (Student's t-test).