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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 antioxidant, anti-inflammatory, 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 flavonoids 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) at the indicated concentrations for 6 h later. TNF-α release was determined by ELISA (R&D Systems, Minneapolis, MN; PeproTech, Rocky Hill, NJ; and eBioscience, San Diego, CA). 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), polynosine polycytidylic acid poly(I:C) (250 μg/ml), flagellin (500 μg/ml), synthetic diacylated lipoprotein Pam2Cys-Ser-Lys4 (20 ng/ml), synthetic CpG oligodeoxynucleotides 1826 (200 nM), and peptidoglycan from *Staphylococcus aureus* (1 μg/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). DCs 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 determined by flow cytometry. C–E, Inhibitory effect of quercetin on DCs treated with the indicated doses of LPS (C), DCs activated with LPS (100 ng/ml) before or after quercetin treatment (D), and DCs stimulated with various TLR ligands, as indicated (E). Data shown were means ± SD of samples from three wells. NS, p > 0.05; *p < 0.05; **p < 0.01 (Student’s t test) was for the comparison between quercetin-treated and -untreated TLR ligand-activated DCs. All data are representative of three to five independent experiments.

**FIGURE 1.** Quercetin efficiently inhibited DC activation. Mouse bone marrow-derived DCs were treated with medium, TLR ligands, TLR ligand + DMSO, or TLR ligands + quercetin (stock solution at 25 mM in DMSO), as indicated. Supernatants were collected 6 h later, and TNF-α production was measured by ELISA. A, Dose-dependent inhibition of the production of TNF-α by quercetin. B, The cytotoxicity of quercetin in DCs without or with LPS was analyzed by Annexin V staining. The percentage of Annexin V+ cells was determined by flow cytometry. C–E, Inhibitory effect of quercetin on DCs treated with the indicated doses of LPS (C), DCs activated with LPS (100 ng/ml) before or after quercetin treatment (D), and DCs stimulated with various TLR ligands, as indicated (E). Data shown were means ± SD of samples from three wells. NS, p > 0.05; *p < 0.05; **p < 0.01 (Student’s t test) was for the comparison between quercetin-treated and -untreated TLR ligand-activated DCs. All data are representative of three to five independent experiments.
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 ≥ 100 μM (Fig. 1B), which confirmed the intrinsic inhibitory effect of quercetin at concentrations ≤ 50 μM. We then examined the efficiency of the inhibitory effect of quercetin. Quercetin blocked the DC activation by LPS at high doses (up to 1 μg/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.

**FIGURE 2.** The cytokines and chemokines released from LPS-stimulated DCs were impaired by quercetin. DCs were treated with LPS (100 ng/ml) in the presence of various doses of quercetin or 0.2% DMSO, as indicated. Supernatants were collected after 24 h (6 h for RANTES). The levels of cytokines (A) and chemokines (B) were determined 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. C, The effect of quercetin on expression of β-actin. The expression level of β-actin was determined by Western blot analysis. All data are representative of three independent experiments.

**FIGURE 3.** Quercetin attenuated the LPS-induced DC maturation. DCs were treated with medium, LPS (100 ng/ml), LPS + 0.2% DMSO, or LPS + quercetin (50 μM) for 16 h. The expression levels of MHC class II, CD40, CD80, and CD86 were determined by flow cytometry. All data shown were gated on CD11c^+ cells. The gray-filled area represents staining with an isotype-matched control Ab. The change of mean fluorescence intensity from LPS to LPS + quercetin was indicated. All data are representative of two to four independent experiments.
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 LN 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 T cells. DCs were loaded with OVA323–339 peptide and stimulated with LPS in the absence or presence of quercetin for 16 h. DCs were then washed to avoid the effect of quercetin on T cells (22) and cocultured with OT-II T cells. LPS-activated DCs promoted T cell proliferation, which was abrogated by quercetin (Fig. 6A). Next, we performed an in vivo recall assay. C57BL/6 mice were immunized with OVA mixed with IFA plus DMSO, quercetin, LPS, LPS plus DMSO, or LPS plus quercetin. After 10 d, draining LN cells were incubated with OVA for 3 d. Consistent with the in vitro results, quercetin significantly inhibited T cell proliferation in response to OVA (Fig. 6B). Furthermore, quercetin decreased the production of IFN-γ by activated T cells, both in vitro and in vivo (Fig. 6C). These results demonstrate that quercetin abrogates the ability of DCs to induce Ag-specific T cell activation. The abrogation of recall responses by quercetin in vivo is in agreement with the defect of DC maturation, endocytosis, and migration after quercetin treatment (Figs. 3–5).

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.

The activation of ERK, JNK, Akt, and NF-κB in LPS-stimulated DCs was reduced by quercetin

TLR ligation induces the activation of the MAPK, Akt, and NF-κB pathways, resulting in DC activation. To explore the molecular mechanism by which quercetin develops its inhibitory effect, we investigated which signal pathway is altered by quercetin in activated DCs. ERK, JNK, p38 MAPK, and Akt were activated in DCs after LPS stimulation; however, quercetin blocked the LPS-induced activation of ERK, JNK, and Akt but not of p38 MAPK, whereas the levels of these proteins were not affected.

FIGURE 5. The migration of LPS-stimulated DCs was diminished by quercetin. DCs were treated with medium, LPS (100 ng/ml), LPS + 0.2% DMSO, or LPS + quercetin (50 μM) for 16 h. A, DCs were transferred into the upper wells of a 24-well Transwell chamber, and CCL21 was included in the lower wells. After 3 h, the number of cells that transferred from the upper to the lower wells was counted by flow cytometry. B, DCs were labeled with CFSE and then injected into mouse footpads. The inguinal LN cells were isolated after 2 d, and CFSE− cells were determined by hemocytometry and flow cytometry. Data shown were means ± SD of samples from three wells (A) or three mice (B). *p < 0.01 (Student t test) was for the comparison between quercetin-treated and -untreated LPS-activated DCs. All data are representative of three independent experiments.

FIGURE 4. Quercetin blocked the endocytosis by DCs. DCs were treated with medium, 0.2% DMSO, quercetin (50 μM), PBS, LPS (100 ng/ml) for 1 h (A), or LPS for 16 h (B) and then incubated with dextran-FITC. The gray-filled area represents the cells incubated with dextran-FITC at 4°C as a control. The change of percentage of dextran-FITC+ cells from untreated to treatment was indicated. All data are representative of four independent experiments.
In addition, the I-kB degradation was decreased, indicating that the activity of NF-kB was reduced by quercetin (Fig. 8). These results suggest that quercetin suppresses DC activation via disruption of the ERK, JNK, Akt, and NF-kB 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 block 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 this process (34). Our data showed that quercetin decreased the migration of DCs in a CCR7-dependent manner (Fig. 5). The mechanism underlying this effect is not yet fully understood and warrants further investigation.

**CONCLUSIONS**

In summary, this study provides strong evidence that quercetin is a potent immunosuppressor of DCs. The inhibitory effect of quercetin on DC function is mediated by multiple mechanisms, including disruption of the ERK, JNK, Akt, and NF-kB pathways, and reduction of endocytosis. These findings suggest that quercetin could be a promising agent for the prevention and treatment of inflammatory and autoimmune diseases. Further studies are needed to elucidate the mechanisms underlying the inhibitory effect of quercetin on DC function and to explore the potential of quercetin as a therapeutic agent in the treatment of inflammatory and autoimmune diseases.
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 blockage 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 adapter 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 activation induces regulatory T cell differentiation (45) and suppression of macrophage activation by quercetin. These pathways are consistent with what was observed in macrophages (36, 37). 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 adapter 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.

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Disclosures
The authors have no financial conflicts of interest.

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