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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. Flavonoids are natural products from many vegetables, fruits, herbs, flowers, seeds, and beverages that are present in diet. 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. In addition, a wide range of biological activities has been discussed for several decades. In addition, quercetin exerts antimicrobial, antihyper- tension, neuroprotective, and chemoprotective effects. The immunomodulatory activity of quercetin has been investigated in NK cells, macrophages, mast cells, neutrophils, B cells, and T cells. Although a number of studies have reported the activity of flavonoids in the modulation of DC activation, 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. 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.

Abbreviations used in this paper: AhR, aryl hydrocarbon receptor; CHS, contact hypersensitivity; DC, dendritic cell; DNFB, 2,4-dinitro-1-fluorobenzene; LN, lymph node.
**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 LPS (100 ng/ml), quercetin (50 μM), LPS plus DMSO (0.2%), or LPS plus quercetin for 16 h. Cells were then washed with media 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/OT II 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-
function is not known. Therefore, we first tested whether quercetin 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.
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 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.
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 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 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 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 by which quercetin suppresses DC activation and function in these disease models. Moreover, AhR activation causes DC-mediated islet allograft-specific tolerance (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 nanotechnical encapsulation (57) and that liposomes can stably and efficiently deliver Ag and quercetin for the Ag-specific suppression of inflammatory arthritis (58). Conceivably, these techniques will hasten the development of the clinical applications of quercetin.


