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The flavonoid, quercetin, differentially regulates Th-1 (IFNγ) and Th-2 (IL4) cytokine gene expression by normal peripheral blood mononuclear cells

Madhavan P.N. Nair[a,*], Chithan Kandaswami[b], Supriya Mahajan[a], Kailash C. Chadha[c], Ram Chawda[a], Harikrishnan Nair[a], Niranjan Kumar[d], Raji E. Nair[a], Stanley A. Schwartz[a]

[a] *Departments of Medicine and Microbiology, Kaleida Health System, Buffalo General Hospital, Buffalo, NY 14203, USA*
[b] *Department of Clinical Laboratory Science, State University of New York at Buffalo, Buffalo, NY 14203, USA*
[c] *Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*
[d] *Aventis Pasteur Inc., Swiftwater, PA 18370, USA*

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Abstract

Flavonoids are plant metabolites that are dietary antioxidants and exert significant anti-tumor, anti-allergic, anti-inflammatory and anti-viral effects. It is generally accepted that Th-1 derived cytokines such as IL-2, IFNγ and IL-12 promote cellular immunity while Th-2 derived cytokines such as IL-4, IL-5, IL-6 exert negative immunoregulatory effects on cellular immunity while upregulating humoral immunity. The molecular mechanisms underlying the biological activities of flavonoids have not been elucidated. We hypothesize that the flavonoid, quercetin, exert significant anti-viral and anti-tumor effects possibly by modulating the production of Th-1 and Th-2 derived cytokines. Peripheral blood mononuclear cells (PBMC, 1×10^6 cells/ml) from normal subjects were cultured with different concentrations of quercetin (0.5–50 μM) for 24–72 h and supernates were quantitated for IFN-γ and IL-4 by ELISA and antiviral activity of IFNγ by bioassay. FACS analysis was done to determine the number of IFN-γ and IL-4 positive cells and RT-PCR was done to quantitate gene expression. Quercetin significantly induces the gene expression as well as the production of Th-1 derived IFNγ and the downregulates Th-2 derived IL-4 by normal PBMC. Further, quercetin treatment increased the phenotypic expression of IFNγ cells and decreased IL-4 positive cells by FACS analysis, which corroborate with protein secretion and gene expression studies. These results suggest that the beneficial immuno-stimulatory effects of quercetin may be mediated through the induction of Th-1 derived cytokine, IFNγ, and inhibition of Th-2 derived cytokine, IL-4.

Keywords: Flavonoid; Cytokine; Peripheral blood mononuclear cell

1. Introduction

Food-derived flavonoids, in particular quercetin, critically modulates a variety of inflammatory processes and immune functions and these have been extensively reviewed [1–3]. Recent studies show that Th-1 derived cytokines such as IL-2, IFNγ, and IL-12 promote cellular immunity, while the Th-2 derived cytokines such as IL-4, IL-5, and IL-6 on the other hand, exert negative immunoregulatory effects on cellular immunity, while upregulating humoral immunity. In addition to the primary anti-viral and anti-cancer effects, the biological functions of the Th-1 derived cytokine, IFNγ, include differentiation of B cells, activation of MHC class I and II molecules, inhibition of Th-2 cell growth and activation of NK cells (review Ref. [4]). Its main antiviral activity includes the elimination or blocking of viral replication in infected cells. Th-2 derived cytokine, IL-4, is primarily involved in the activation of B cells, promotion of growth and survival of T cells, inhibition of macrophage activation and suppression of Th-1 cells. Recent studies show that IL-4 and IFNγ play a significant role in the regulation of immune responses by their mutually antagonistic mechanisms (review Ref. [5]). Although previous studies suggest that quercetin exhibits significant anti-tumor, anti-viral, anti-inflammatory, and anti-allergic properties [1–11], the molecular mechanisms of their biological responses remain to be delineated. We hypothesize

* Corresponding author. Division of Allergy, Immunology and Rheumatology, Department of Medicine, Buffalo General Hospital, 310 Multi Research Bldg., 100 High Street, Buffalo, NY 14203, USA.
Tel.: +1-716-859-2985; fax: +1-716-859-2999.
E-mail address: mnair@acsu.buffalo.edu (M.P.N. Nair).

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that flavonoids exert significant immunomodulatory effects by modulating Th-1 and Th-2 derived cytokines. The present study was undertaken to investigate the direct effect of quercetin on Th-1 derived cytokine (IFNγ) and Th-2 derived cytokine (IL-4) gene expression, protein secretion as well as phenotypic expression of IFN γ and IL-4 positive cells in normal peripheral blood mononuclear cells (PBMC).

2. Materials and methods

2.1. Cell culture

PBMCs (1×10^6 cells/ml) were cultured in RPMI1640 medium containing 10% fetal bovine serum (FBS, complete medium) with quercetin at concentrations ranging between 0.5 and 50 μM for 24–72 h at 37 °C in 5% CO^2 incubator. The supernatants were harvested and stored at − 70 °C until further analysis for ELISA and antiviral effects. The cells were analyzed by flow cytometry while RNA was extracted from the cells for RT-PCR.

2.2. RNA extraction

Cytoplasmic RNA was extracted by an acid guanidinium thiocyanate–phenol chloroform method as described by Chomczynski and Sacchi [12]. Cultured cells were pelleted by centrifugation and resuspended in a 4 M solution of guanidinium thiocyanate. Cells were agitated by repeated pipetting so as to lyse them and then phenol–

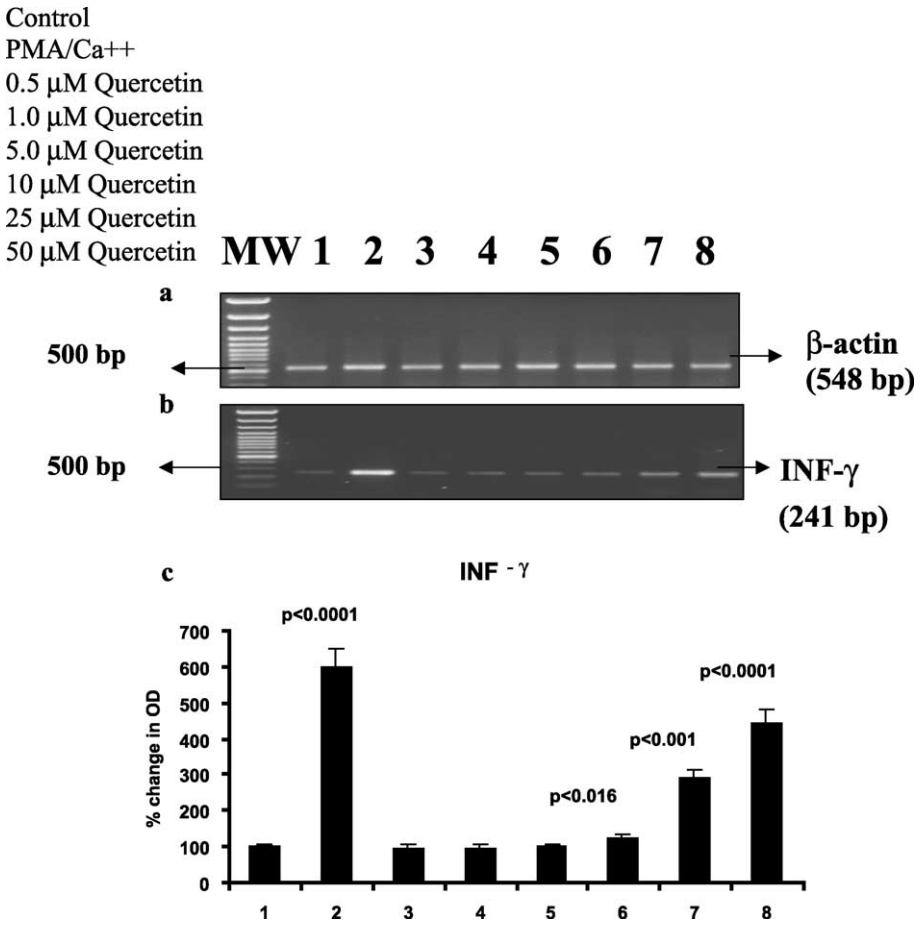

Fig. 1. Quercetin induces IFN-γ gene expression by PBMC as measured by RT-PCR. PBMCs (1×10^6 cells/ml) were cultured in RPMI 1640 medium containing 5% FBS (GIBCO), 300 μg/ml of fresh glutamin and 80 μg/ml of gentamicin (complete medium) alone or with PMA plus Ca^{2+} ionophore (50 ng/ml each) or quercetin at concentrations of 0.5–50 μM (Sigma Chemical, St. Louis, MO) for 24 h. Cytoplasmic mRNA was extracted, reverse-transcribed and amplified with IFN-γ [5′-GAGTGTGGAGACCATCAAGGAA-3′ (upstream), 5′-GCAGGCAGGACAACCATTACTG-3′ (downstream) (241 bp)] and housekeeping β actin [5′-GTGGGGCGCCCCAGGCACCA-3′ (upstream), 5′-CTCCTTAATGTCACGCACGATTTC-3′ (downstream) (548 bp)] specific primers. A 10 μl sample of each of the PCR reactions was analyzed on 1.2% agarose gels containing ethidium bromide with a phi X174 DNA/*Hae* III digested molecular weight standard to determine the fragment sizes. Bands were visualized under UV light and photographed using Polaroid film. cDNA from amplified PCR products of β-actin (a), and specific IFNγ (b) banded at 548 and 241 bp, respectively. (c) Quantitation of changes in IFNγ gene expression. Percent changes in laser densitometry reading of the photographic negatives of experimental values after normalization with corresponding β-actin values were compared with control values. This data represents mean ± S.D. of three experiments using PBMC from three different subjects. The statistical significance of the difference between control and treated samples was calculated by Student's *t*-test. In kinetic studies, quercetin-induced effect was evident at 24 h and the effect tends to decrease at 48 and 72 h (data not provided).

Table 1
Effect of quercetin on INF-γ induction by PBMC

Treatment of PBMC	INF-γ (units)
0.5 μM Quercetin	0
1.0 μM Quercetin	0
5.0 μM Quercetin	4.5 ± 0.707 ($P<0.035$)
10 μM Quercetin	6.5 ± 2.10 ($P<0.072$)
25 μM Quercetin	10.25 ± 0.012 ($P<0.007$)
50 μM Quercetin	23.01 ± 4.24 ($P<0.041$)

Quercetin induces IFNγ antiviral activity by normal PBMC. PBMCs (1×10^6 cells/ml) were cultured alone or with PMA plus Ca^{2+} (50 ng/ml each) or different concentrations of quercetin (0.5–50 μM) for 48 h. Supernates were assayed for IFNγ antiviral activity in a bioassay using BG-9 cells against VSV as challenging virus. Data are mean ± S.D. of three experiments performed in triplicates using PBMC from three different subjects. Statistical significance of the differences between control and quercetin-treated cultures was evaluated by Student's *t*-test.

chloroform-extracted in the presence of sodium acetate. After centrifugation, RNA was precipitated from the aqueous layer by adding an equal volume of isopropanol and the mixture was kept at −20 °C for 1 h and then centrifuged to pellet the RNA. The RNA pellet was washed with 75% ethanol to remove any remaining traces of guanidium. The final pellet was dried and resuspended in DEPC water and the amount of RNA determined using a spectrophotometer at 260 nm. Isolated RNA was kept at −70 °C until used.

2.3. RT-PCR

The extracted RNA was used for RT-PCR. The RT-PCR reactions were performed using a Perkin Elmer kit (Cat # N808-0143). The RNA was reverse-transcribed to make a DNA copy for use in PCR. Briefly, 1 μg of RNA was added to a tube containing 5 mM $MgCl_2$, 1 mM each of dNTP (ATGC), 50 mM KCl, 10 mM Tris buffer pH 8.3, 2.5 μM oligodT, 20 units of RNase-inhibitor and 50 units of MuLV reverse transcriptase. The mixture was incubated at 45 °C for 35 min and then heated to 95 °C for 5 min and placed on ice until used for PCR. This newly synthesized cDNA was then amplified by PCR using specific sense and antisense primers of the genes of interest along with a housekeeping gene, β-actin, as a control. To each tube, a 10-μl sample of the RT product in a final concentration of 2 mM $MgCl_2$, 10 mM Tris pH 8.3, 50 mM KCl, plus 0.02 μM of both the 5′ and 3′ primers and 2.5 units of Taq polymerase was added. The mixture was then placed in a thermocycler for 30 cycles of 95 °C for 30 s, 60 °C for 30 s and 74 °C for 1 min. The PCR conditions were modified slightly for INF-γ and IL-4 genes to obtain optimal results. Samples are then separated on a 1–1.2% agarose gel along with a molecular weight marker for reference, and bands were visualized, fragment size determined and quantitated using a scanning densitometer. All values were normalized to the constitutive expression of the housekeeping gene.

2.4. Interferon-γ: antiviral assays

2.4.1. Cells and viruses

Primary human foreskin fibroblast (BG-9) cells were used for assay of interferon antiviral activity using vesicular stomatitis virus (VSV) as a challenge virus. BG-9 cells were maintained in Minimal Essential Medium containing non-essential amino acids, 10% heat-inactivated FBS, 100 U/ml penicillin and 100 μl streptomycin in a 37 °C and 5% CO_2 atmosphere. VSV was grown and quantitated by plaque assay on CV-1 cells.

2.4.2. Interferon gamma (HuIFN-γ) production

PBMCs (1×10^6 cells/ml) in RPMI 1640 complete medium were incubated with quercetin at different concentrations or with PMA/Ca^{2+} alone (50 ng/ml each) for IFNγ production. The supernatant fluid was collected from 48-h culture by low speed centrifugation and dialyzed against PBS prior to assay for IFN-γ antiviral activity.

2.4.3. Interferon antiviral assays

Antiviral activity for IFNγ was assayed on BG-9 cells by the dye uptake using VSV as a challenge virus method [13]. Briefly, confluent monolayers of BG-9 cells in 96-well tissue culture trays were exposed to a serial two-fold dilutions of interferon preparations. Twenty-four hours later, the monolayers were washed once with warm medium and then challenged with VSV. After lysis of control cells (approximately 36 h), the trays were stained with neutral red dye (40 μg/ml). The dye taken up by interferon protected cells was extracted with a solution containing 50% absolute ethanol, 45% distilled water and 1% glacial acetic acid and quantitated spectrophotometrically at 540 nm. All titers are expressed as International reference units (IU). Reference standard was kindly provided by the National Research Resource Branch of the National Institute of Allergy and

Table 2
Effect of quercetin on INF-γ induction by PBMC

Treatment of PBMC	INF-γ (pg/ml)
Control	0
PMA/Ca^{2+}	1330.67 ± 44.40 ($P<0.000001$)
0.5 μM Quercetin	0
1.0 μM Quercetin	0
5.0 μM Quercetin	4.58 ± 2.83 ($P<0.0013$)
10 μM Quercetin	14.04 ± 5.54 ($P<0.00005$)
25 μM Quercetin	43.81 ± 19.53 ($P<0.0001$)
50 μM Quercetin	575.75 ± 67.78 ($P<0.000001$)

Quercetin induces IFNγ production by normal PBMC. PBMCs (1×10^6 cells/ml) were cultured alone or with PMA plus Ca^{2+} (50 ng/ml each) or different concentrations of quercetin (0.5–50 μM) for 48 h. Supernates were assayed for IFNγ by a quantitative sandwich enzyme immunoassay technique using the Cytoscreen Immunoassay Kit (BioSource). Data are mean ± S.D. of three experiments performed in triplicates using PBMC from three different subjects. Statistical significance of the differences between control and quercetin-treated cultures was evaluated by Students *t*-test. The sensitivity of the assay is <4 pg/ml.

Infectious Diseases, Bethesda, MD. The identity of INF-γ activity was confirmed by both antibody neutralization and by the pH2 sensitivity assays.

2.5. FACS analysis

Immunofluorescent staining was used to identify and quantitate the number of cells that express intracellular cytokines IL-4 and INF-γ in response to quercetin treatment as recommended by the manufacturers of monoclonal antibodies.

Approximately 1×10^6 lymphocytes were treated with quercetin (1, 5, 25, and 50 μM) for 24 h After 24-h incubation, cells were harvested, washed and suspended in staining buffer. We used golgi stop (BD Pharmingen), an intracellular protein transport inhibitor that helps to enhance the ability to detect cytokine-producing cells. FACS Calibur conditions were optimized on adjusting the settings for PMT voltage and compensation. Using appropriate controls, the quadrant markers were set using specified isotype controls for each flurochrome-conjugated specific antibody used. The mAbs were conjugated to PE/FITC. Matched isotype controls were obtained from BD Pharmingen. Cells were fixed by resuspending them in 100 μl of cytofix/cytoperm buffer for 10–20 min at 4 °C. Cells were then permeabilised by washing twice in 1× Perm solution and then resuspended in staining buffer prior to flow cytometric analysis. Stained cells were subjected to a light scatter analysis and a fixed population of cells was gated when represented as side scatter (SSC) on *y*-axis and forward scatter (FSC) on *x*-axis. Cells positive for IL-4 and INF-γ were expressed as a percentage of the total cells gated.

2.6. ELISA

The INF-γ and IL-4 protein secretion in culture supernatants were quantitated using highly specific and ultrasensitive ELISA kits obtained from BioSource International

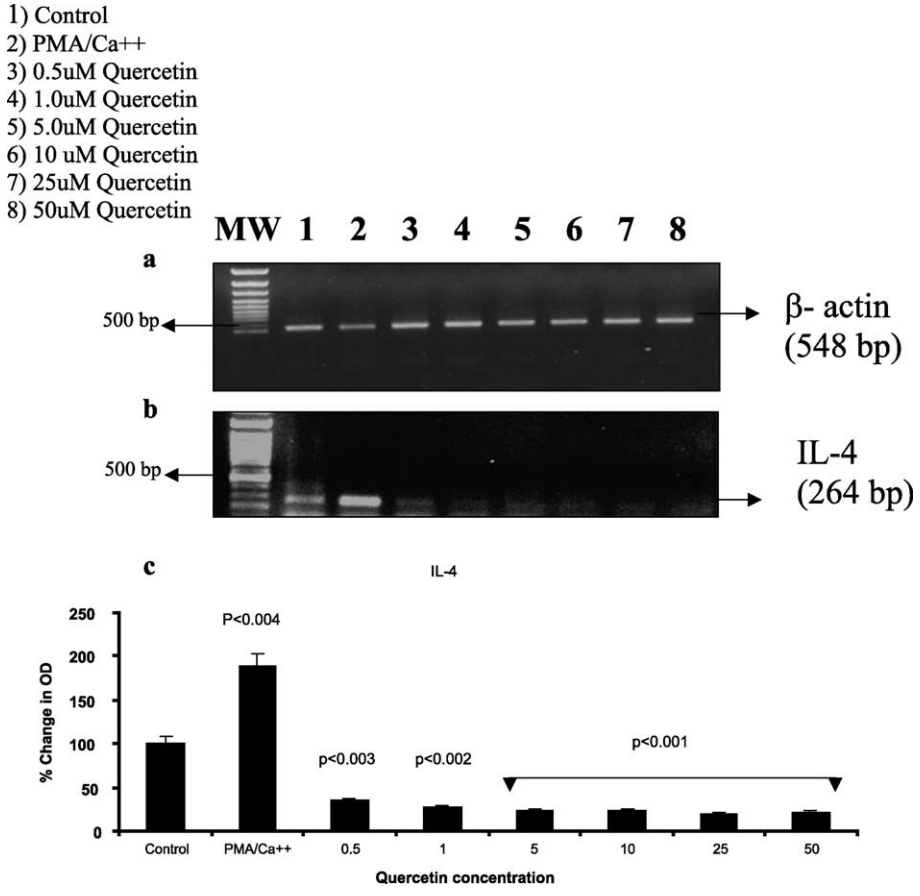

Fig. 2. Quercetin suppresses IL-4 gene expression by PBMC as measured by RT-PCR. PBMCs (1×10^6 cells/ml) were cultured in medium alone or with quercetin at concentrations of 0.5–50 mM (Sigma) for 24 h. The RT-PCR assays were performed using the Perkin-Elmer kit (Cat. #N808-0143). The newly synthesized cDNA products were used with IL-4 [5′ TGC TGC CTC CAA GAA CAC AAC TG (upstream), 3′-CAT GAT CGT CTT TAG CCT TTC CA (downstream) (264 bp)] and β-actin specific primers as used for Fig. 1 in a PCR assay using 37 cycles. cDNA from amplified PCR products of β-actin and specific IL-4 banded at 548 bp (a) and 264 bp (b), respectively. (c) Quantitation of changes in IL-4 gene expression. Percent changes in laser densitometry reading of the photographic negatives of experimental values after normalization with corresponding β-actin values were compared with control values. This data represents mean ± S.D. of three separate experiments using PBMC from three different subjects. Statistical significance of the differences between control and quercetin-treated cultures was evaluated by Student's *t*-test.

Table 3
Effect of Quercetin on IL-4 induction by PBMC

Treatment of PBMC	IL-4 (pg/ml)
Control	4.66 ± 0.49
PMA/Ca^{2+}	442.89 ± 7.50 ($P<0.0001$)
0.5 μM Quercetin	0.902 ± 0.39 ($P<0.014$)
1.0 μM Quercetin	0.270 ± 0.061 ($P<0.006$)
5.0 μM Quercetin	0
10 μM Quercetin	0
25 μM Quercetin	0
50 μM Quercetin	0

Quercetin inhibits IL-4 production by normal PBMC. PBMCs (1×10^6 cells/ml) were cultured alone or with PMA plus Ca^{2+} (50 ng/ml each) or different concentrations of quercetin (0.5–50 μM) for 48 h. Supernates were assayed for IL-4 by a quantitative sandwich enzyme immunoassay technique using the Cytoscreen Immunoassay Kit (BioSource). Data are mean ± S.D. of three experiments performed in triplicates using PBMC from three different subjects. Statistical significance of the differences between control and quercetin-treated cultures was evaluated by Student's *t*-test. The sensitivity of the assay is <0.1 pg/ml.

(Camarillo, CA) and used as described by the manufacturer. The sensitivity of the IFN-γ and IL-4 ELISA were 4 and 0.1 pg/ml, respectively.

3. Results

3.1. Quercetin upregulates IFN-γ gene expression and production

Data presented in Fig. 1 show the effect of quercetin on IFNγ gene expression by PBMC. PCR products of housekeeping β-actin (Fig. 1a) and specific IFN-γ (Fig. 1b) migrated to the expected region of 548 and 241 bp, respectively. Quercetin did not affect housekeeping gene expression (Fig. 1a, lanes 2–8) and was comparable to control culture (Fig. 1a, lane 1). PMA plus Ca^{2+} at 50 ng/ml each used as a positive stimulant significantly increased the IFNγ gene expression (lane 2, OD = 1.3, 495% enhancement) compared to control culture (lane 1, OD = 0.21). Quercetin at 10 μM (lane 6, OD = 0.26, 20% enhancement) 25 μM (lane 7, OD = 0.51, 138% enhancement) and 50 μM (lane, 8, OD = 0.86, 301% enhancement) significantly upregulated IFNγ gene expression compared to control culture (lane 1, OD = 0.21). Quercetin at lower concentrations of 0.5 μM (lane 3), 1 μM (lane 4) and 5 μM (lane 5) did not show any modulation of IFNγ gene expression and was comparable to

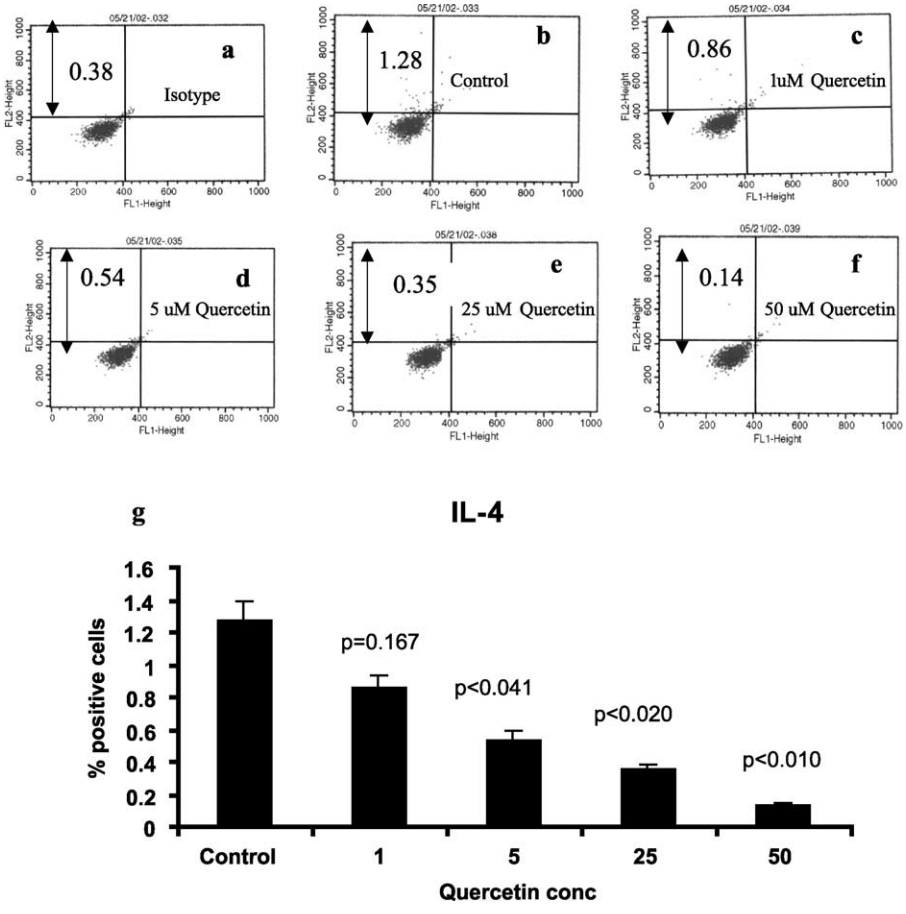

Fig. 3. Quercetin suppresses IL-4 positive cells as measured by flow cytometry analysis. FACS analysis to quantitate IL-4 positive cells in cultures treated with quercetin was performed as described in Materials and methods. PE labeled isotype control antibody (IgG2b) was used a negative control (a) and to set quadrants. (b) Control culture. (c–f) Cultures treated with quercetin at 1, 5, 25, and 50 μM respectively. (g) Mean ± S.D. of three separate experiments. Statistical significance of the difference between control and treated cultures was evaluated by Student's *t*-test.

control culture (lane 1). Data presented in Fig. 1c show the mean percent changes ± S.D. in OD values from three separate experiments performed similarly as shown in Fig. 1a,b. Quercetin at 10 μM ($P<0.016$) 25 μM ($P<0.001$) and 50 μM ($P<0.0001$) concentrations significantly upregulated IFNγ gene expression compared to control culture (lane 1).

Supernates from PBMC cultures treated with quercetin were assayed for their IFNγ antiviral activity in bioassay using BG6 cells against VSV as challenge virus. PBMC treated with PMA plus Ca^{2+} alone (positive control) produced 65 units of IFN-γ activity compared to 0 unit activity produced by control culture (Table 1) Querectin at 5, 10, 25, and 50 μM concentration produced, respectively, 4 ($P<0.03$) 6 ($P<0.007$), 10 ($P<0.007$) and 23 ($P<0.04$) units of IFN-γ activity as compared to 0 unit activity manifested by control culture. Quercetin at lower concentrations of 0.5 and 1 μM produced no antiviral activity and was comparable to control culture. We also measured the levels of IFNγ in the culture supernates of PBMC treated with different concentrations of quercetin by ELISA. PMA plus Ca^{2+} (50 ng/ml each) used as a positive stimulant significantly increased the IFNγ production (1330 pg/ml, $P<0.000001$) compared to control culture (0 pg/ml). Quercetin at 5, 10, 25 and 50 μM produced significantly higher levels of IFNγ compared to control culture. The IFNγ levels being 4 pg/ml ($P<0.001$), 14 pg/ml ($P<0.00005$), 43 pg/ml ($P<0.0001$), and 575 pg/ml ($P<0.000001$), respectively, compared to 0 levels of IFNγ produced by the control culture. Thus, the quantitation of IFNγ by ELISA (Table 2) and the measurement of interferon activity in bioassay (Table 1) are consistent with our gene expression data as analyzed by RT-PCR (Fig. 1). This suggests that IFNγ can be induced by quercetin, which may be of clinical significance in host defense mechanisms against various infections.

3.2. Quercetin inhibits IL-4 gene expression in normal PBMC

We also studied the effects of quercetin on Th-2 derived cytokine, IL-4, gene expression by PBMC (Fig. 2). In the mRNA analysis at 24 h, quercetin significantly inhibited IL-4 specific gene expression. Quercetin treatment (Fig. 2a, lanes 3–8) did not affect the housekeeping β-actin-specific RNA gene expression and was comparable to control

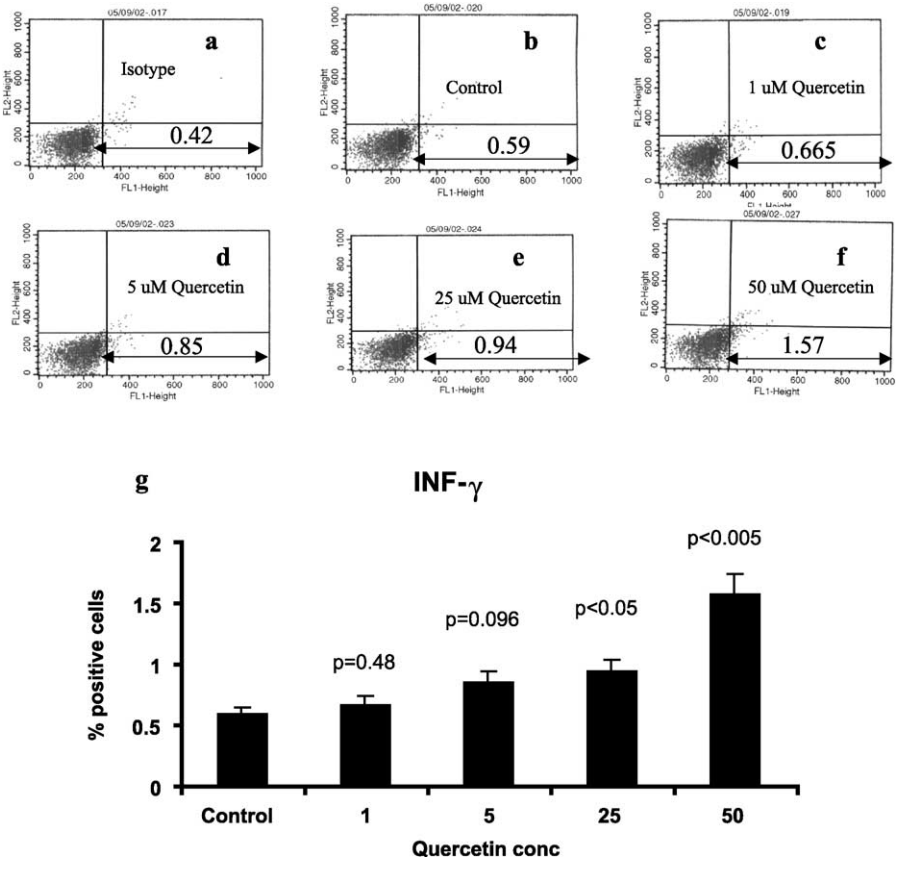

Fig. 4. Quercetin increases IFN-γ positive cells as measured by flow cytometry analysis. FACS analysis to quantitate IFN-γ positive cells in cultures treated with quercetin was performed as described in Materials and methods. FITC labeled isotype control antibody (IgG2b) was used a negative control (a) and to set quadrants. (b) Control culture. (c–f) Cultures treated with quercetin at 1, 5, 25, and 50 μM, respectively. (g) Mean ± S.D. of three separate experiments. Statistical significance of the difference between control and treated cultures was evaluated by Student's *t*-test.

culture (lane 1). PMA plus Ca^{2+} at 50 ng/ml each used as a positive stimulant significantly increased the IL-4 gene expression (lane 2, OD = 1.57, 89% enhancement) compared to control culture (lane 1, OD = 0.83). Cultures treated with quercetin at 0.5 μM (OD = 0.28, 66% inhibition) and 1 μM (OD = 0.28, 72% inhibition) significantly inhibited the endogenous IL-4 gene expression compared to control culture (lane 1; OD = 0.83). Quercetin at 5 μM (lane 5; OD = 0.19, 77% inhibition), 10 μM (lane 6; OD = 0.187, 78%), 25 μM (lane 7; OD = 0.181, 78.5% inhibition), and 50 μM (lane 8; OD = 0.16, 80% inhibition) concentrations showed significant suppression of IL4 gene expression compared to control culture (lane 1; OD = 0.83). Data presented in Fig. 2c show the mean percent changes ± S.D. S.D. in OD values from three separate experiments performed similarly as shown in Fig. 2a,b. Quercetin at 0.5 μM ($P < 0.003$), 1 μM ($P < 0.002$), 5 μM ($P < 0.001$), 10 μM ($P < 0.001$), 25 μM ($P < 0.001$), and 50 μM ($P < 0.001$) concentrations significantly inhibited endogenous IL-4 gene expression compared to control culture (lane 1).

We also studied the effects of quercetin on endogenous production of IL-4 by PBMC as quantitated by ELISA. PMA plus Ca^{2+} (50 ng/ml each) used as a positive stimulant significantly induced the IL-4 production (442 pg/ml, $P < 0.0001$) compared to a negligible level of IL-4 produced by control culture (4.6 pg/ml). Quercetin at 0.5 μM (0.9 pg/ml, $P < 0.014$) and 1 μM (0.27 pg/ml, $P < 0.006$) significantly inhibited the endogenous levels of IL-4 compared to control culture. Quercetin at increased concentrations of 5, 10, 25, and 50 μM completely inhibited the endogenous levels of IL-4 produced by PBMC. Thus, the quantitation of IL-4 by ELISA (Table 3) is consistent with our gene expression data as analyzed by RT-PCR (Fig. 2). This suggests that Th-2 derived cytokine, IL-4, can be inhibited by quercetin, which may be of clinical significance in host defense mechanisms against various infections.

3.3. Quercetin modulates IL-4 and INF-γ positive phenotypes

Data presented in Figs. 3 and 4, respectively, show the effect of quercetin on intracellular markers, IL-4 and INF-γ, as demonstrated by flow cytometry analysis. PBMC treated with 5.25 and 50 μM quercetin demonstrated a significant decrease in the percentage of IL-4 positive cells (Fig. 3d–f, respectively), while PBMC treated with 25 and 50 mM showed significant increase in the percentage of INF-γ positive cells (Fig. 4e and f, respectively). Figs. 3g and 4g show the mean percent ± S.D. of IL-4 and IFNγ positive cells, respectively, for three separate experiments and quercetin showed a dose-dependent decrease in IL-4 positive cells and dose-dependent increase in IFNγ positive cells compared to control culture. These results confirm our gene expression data as analyzed by RT-PCR for IFNγ (Fig. 1) and IL-4 (Fig. 2), respectively.

4. Discussion

In general, studies dealing with the absorption and bioavailability of quercetin in human subjects have yielded inconsistent and widely divergent results. Previous studies have shown a high absorption of quercetin from the intestine following consumption of onion quercetin glycosides [14]. Recent studies have also reported mean plasma concentrations of quercetin is 5 μM in human subjects following the consumption of quercetin glucosides [15]. In the current studies, quercetin at as low a concentration as 5 μM, similar to plasma levels, show a significant effect on IFNγ and IL-4 gene expression. Therefore, these studies may be of physiological and clinical relevance.

It is currently recognized that helper T lymphocytes (Th) may be divided into two functional subclasses, Th-1 and Th-2 cells, based upon the cytokines that they produce and their effects on cell mediated and humoral immunity. Th-1 cells produce interleukin 2, IFNγ, and IL-12 and enhance cell-mediated immunity. Th-1 cells also can inhibit cell-mediated immunologic activities. Th-2 cells produce IL-4, IL-5, IL-6 and IL-10 and upregulate humoral immunity. In addition, Th-1 and Th-2 derived cytokines cross-regulate each other in various clinical conditions.In our studies, quercetin induced the production and gene expression of Th-1 derived cytokine, IFNγ, as measured by its antiviral activity assay, flow cytometry, and RT-PCR. However, quercetin significantly downregulated Th-2 derived cytokine, IL-4, gene expression as well as IL-4 production by PBMC. The differentiation of precursor Th-0 cells in to Th-1 and Th-2 subsets depend on whether IFNγ or IL-4 is present during the differentiation [16], and our studies show that quercetin can induce IFNγ favoring a Th-1-mediated immune reaction while inhibiting Th-2 or humoral immune response. We also demonstrated that this effect is biologically significant as quercetin can induce an increase in lymphocyte with intracytoplasmic IFNγ as well as synthesis and secretion of IFNγ. Thus, these two cytokines play a cardinal role in the regulation of various immune responses. The current studies show for the first time that quercetin can downregulate IL-4 and upregulate IFN-γ gene expression. Previous studies show that IFNγ can downregulate IL-4 [17], and, therefore, it is possible that suppression of IL-4, as observed in the current investigation, may be partially mediated by quercetin-induced IFN-γ in our culture.

Previous studies [6] show that antiviral activity of TNF against VSV and encephalomyocarditis virus (EMCV) is greatly enhanced by quercetin in WISH cells and polyclonal antibodies to interferon blocked the antiviral activity, which suggests that quercetin-induced antiviral activity may be mediated through induction of interferon. Our studies show a direct evidence of IFNγ production by quercetin and support the notion that antiviral activity of quercetin may be mediated through IFNγ production.

The direct inhibitory effect of quercetin on IL-4 gene expression and production without the potential regulatory

effect of IFN-γ needs to be investigated. Further, the inhibitory effects of quercetin on IL-4 gene expression were not due to the toxicity of quercetin on PBMC, because PBMC treated with quercetin concentration as high as 50 μM after 96 h incubation yielded almost 90% viable cells comparable to control cultures. Quercetin-mediated differentiation of Th-2 to Th-1 cell types or participation by any other cytokines or immunoregulatoy molecules in the preferential upregulation of IFN-γ and downregulation of IL-4 genes need to be studied. Evaluation of the molecular mechanisms of quercetin-mediated immunomodulatory effects may be a promising area for the development of new flavonoid-based neutrapharmaceutical agents for the treatment of certain immune-mediated diseases and viral infections.

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