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Expression Profiling of Genes Targeted by Bilberry (*Vaccinium myrtillus*) in Macrophages Through DNA Microarray

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To evaluate the anti-inflammatory function and underlying genes targeted by bilberry, gene expression profiling through DNA microarray was performed on bilberry extract-treated macrophages. Among 22,050 oligonucleotides, the expression levels of 998 genes were increased by ≥ twofold in lipopolysaccharide (LPS)-activated RAW264 cells, 358 gene signals of which were attenuated by bilberry extract (≥ 1.5-fold). Expression levels of 2,086 genes were decreased by ≥ twofold in LPS-activated cells, of which 939 gene signals were enhanced by bilberry extract (≥ 1.5-fold). Utilizing Panther group analysis, 308 genes affected by bilberry extract were classified into 43 categories relating to biological processes (97), molecular functions (186), and signaling pathways (26) with ≥ 1.5-fold change. The genes categorized as "defense, inflammatory response, cytokines activities, and receptor activities" were further identified, and some of them were confirmed by real-time polymerase chain reaction. The DNA microarray results provide a molecular basis for the anti-inflammatory effects of bilberry.

INTRODUCTION

Anthocyanins are naturally occurring polyphenolic compounds that give blue, violet, or red color to many fruits and vegetables (1,2). They are present exclusively as glycosidic compounds in plants. Variations in the number and nature of the different attached sugar moieties are responsible for the high number of anthocyanins observed in nature, more than 500 compounds (3). The aglycone forms of anthocyanins (named anthocyanidins) contain a diphenylpropane-based polyphenolic ring structure and are limited to a few structural variants including delphinidin, cyanidin, pelargonidin, peonidin, and malvidin, which represent the aglycones of most anthocyanins found in plants. They are of great nutritional interest because of the marked daily intake (180 to 215 mg/day in the United States), which is much higher than the intake (23 mg/day) estimated for other flavonoids such as quercetin, kaempferol, myricetin, apigenin, and luteolin (4,5). They have been reported to have positive effects in the treatment of various diseases (6) and are prescribed as medicines in many countries. Recently, anthocyanins have received increasing attention as natural phytochemicals for cancer chemoprevention (6–8). Some anthocyanins or their aglycones from blackberry (9) and tart cherries (10) could inhibit cyclooxygenase (COX-2) expression and activity in vitro. Our group recently has reported that anthocyanidins inhibited lipopolysaccharide (LPS)-induced COX-2 expression with a structure-activity relationship (11). The orthodihydroxyphenyl structure on the B-ring of anthocyanidins appears to be essential for their COX-2 inhibitory action, as pelargonidin, peonidin, and malvidin, which lack the orthodihydroxyphenyl structure, failed to show an inhibitory effect. Furthermore, delphinidin suppressed LPS-mediated COX-2 expression by blocking mitogen-activated protein kinase (MAPK) pathways (11). These data suggest that anthocyanins may have potential as anti-inflammatory and chemopreventive compounds.

Inflammation is essential for defense against bacterial infection. Crucial events in the inflammatory processes include expression of inflammatory cytokines, chemokines, and other mediators (12). Recognition of invading pathogens by host cells initiates with the binding of specific cellular receptors to pathogen molecules with distinct patterns (13). The major pattern-recognition receptors identified to date consist of the Toll-like receptors (TLR). Inflammation caused by LPS is 1 of the most extensively studied cases (14). LPS liberated from Gram-negative bacteria associates with LPS binding protein, an acute-phase protein present in the bloodstream, and then binds to CD14, a glycosylphosphatidylinositol-linked protein expressed on the cell surface of phagocytes. LPS is then transferred to MD-2, which associates with the extracellular portion of TLR4, followed by oligomerization of TLR4, a key molecule of LPS signaling (15,16). TLR4 signaling promptly induces potent innate immune responses through adaptor molecules such as myeloid differentiation factor 88 (MyD88), Toll/interleukin (IL)-1 receptor (TIR) domain containing adaptor protein, TIR

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domain containing adaptor inducing interferon (IFN)-β (TRIF)-related adaptor molecule (TRAM), and TRIF to activate intracellular signaling pathways involving IL-1 receptor-associated kinase, tumor necrosis factor (TNF) receptor associated factor (TRAF6), nuclear factor (NF)-κB inhibitor alpha kinase, and MAPKs (17,18). These signaling pathways ultimately stimulate transcription factors, NF-κB, activator protein 1, and interferon regulatory factors to induce antibacterial and antiviral responses including the induction of TNF, IL-1, and IL-6 (19).

DNA microarray technology has greatly enhanced our ability to study and understand the effects and underlying mechanisms of natural bioactive products on genome-wide gene expression level. Moreover, bilberries (*Vaccinium myrtillus*) are 1 of the berries containing the highest levels of anthocyanins, and the extract is commercially available (Mirtoselect, Indena SpA, Milan, Italy) as standardized anthocyanins that contain 15 anthocyanins (20). To evaluate the anti-inflammatory function and underlying genes targeted by bilberry, we investigated the effects of bilberry extract on genome-wide gene expression in an inflammatory cell model, RAW264, using an oligonucleotide DNA microarray. The results suggest the molecular basis for the anti-inflammatory activity of bilberry.

MATERIALS AND METHODS

Materials and Cell Culture

Bilberry extract, Mirtoselect, was obtained from Indena SpA (Milan, Italy), as an extract from *Vaccinium myrtillus* and is composed of approximately 40% of anthocyanins (20). The residual content is comprised of plant-derived polysaccharides. LPS (Escherichia coli Serotype 055:B5) and other reagents used in the chemical analysis were purchased from Sigma Chemical (St. Louis, MO).

Murine macrophage-like RAW264 cells were obtained from RIKEN BioResource Center Cell Bank (Cell No. RCB0535), Tsukuba, Japan, and cultured at 37°C in a 5% carbon dioxide atmosphere in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum.

High-Performance Liquid Chromatography (HPLC) Analysis of Bilberry Anthocyanins

The anthocyanin contents in Mirselect sample was determined by HPLC (JASCO, Tokyo, Japan) using authentic anthocyanins as standards. Separation of anthocyanins was achieved on a CrestPak C18T-5 (4.6 $\phi \times$ 150 nm, 5 μM) at 40°C. The flow rate was 1 ml/min. The column was equilibrated with a solvent containing 85% A (1.5% phosphoric acid) and 15% B (1.5% phosphoric acid, 20% acetic acid, and 25% acetonitrile) for 60 min. Anthocyanins were then eluted with gradient solvents, A and B, at 85–45% A (15–55% B) for 60 min. Spectrophotometric detection was at 520 nm. The results are shown in Fig. 1.

RNA Preparation and Microarray Hybridization

RAW264.7 cells were pretreated with or without bilberry extracts for 30 min and then exposed to 40 ng/ml LPS for 6 h. Total RNA was extracted using Qiagen^{TM}RNeasy Mini Kits (Valencia, CA) following the manufacturer's protocol. RNA quantity was assessed using an Agilent 2100 bioanalyzer (Palo Alto, CA). A total of 500 ng of total RNA were amplified at 40°C for 2 h by Agilent low RNA input fluorescent linear amplification kits following the manufacturer's protocol. The cRNAs were labeled at 40°C for 2 h with cyanine 5(cy5) for samples and with cyanine 3(cy3) for the universal mouse reference RNA (Agilent Technologies). After the amplification and labeling, the yields and dye incorporation efficiencies were determined using a spectrophotometer. Agilent mouse 22,050 60-mer oligonucleotide microarrays were used for this study following Agilent 60-mer oligo microarray processing protocol. Briefly, cyanine (Cy)3-labeled samples and Cy5-labeled references were mixed and incubated with an Agilent microarray slide for 17 h using an Agilent in situ hybridization kit. After washing with stabilization and drying solution (Agilent Technologies), microarrays were scanned in Agilent model G2505A microarray scanner.

Data Analysis

Images were processed using Agilent Feature Extraction software, which provides normalized cy3 and cy5 channel intensity values for each spot on an array. For a detailed description of the Agilent Feature Extraction software and the algorithms, see the *Agilent Feature Extraction User Manual.* The selected genes were further classified into the biological process, molecular function, and signaling pathway by the Panther gene group analysis software (http://www.pantherdb.org/).

Reverse Transcription and Real-Time Polymerase Chain Reaction (PCR)

The primers used in this study are shown in Table 1. The primers for IL-1β, IL-6, interferon-inducible protein 1 (IFI1), and IFI47 were designed according to the National Center for Biotechnology Information sequence database using the software PRIMER3. The primers for TNF (21), COX-2 (19), CC chemokine ligand 22 (CCL22) (22), prostaglandin-endoperoxide synthase 2 (PTSG2) (23), and tenascin C (23) have been described previously. Reverse transcription and real-time PCR were performed with DyNAmo^{TM} SYBR®Green 2-Step qRT-PCR Kit (Finnzymes Oy., Espoo, Finland) according to manufacturer's manual. Briefly, RNA (200 ng) was reversed to cDNA using Oligo dT and M-MuLV RNase at 37°C for 30 min, and the reaction was then terminated at 85°C for 5 min. Quantitative PCR was performed with the Roter-Gene-3000AKAA (Corbett Research, New South Wales, Australia) in triplicates using the standard curve. The temperature melting (Tm) of PCR was determined according to each primer sequence (https://www.finnzymes.fi/tm.determination.html). Each PCR

No.	Anthocyanins	Abb	R.T.(min)	Area(%)
1	Delphinidin 3-galactoside	Dp 3-Gal	9.83	12.31
2	Delphinidin 3-glucoside	Dp 3-Glc	11.45	13.13
3	Cyanidin 3-galactoside	Cy 3-Gal	13.16	10.06
4	Delphinidin 3-arabinoside	Dp 3-Ara	13.72	11.27
5	Cyanidin 3-glucoside	Cy 3-Glc	15.41	10.58
6	Petunidin 3-galactoside	Pt 3-Gal	16.24	4.23
7	Cyanidin 3-arabinoside	Cy 3-Ara	17.68	8.01
8	Petunidin 3-glucoside	Pt 3-Glc	18.64	7.91
9	Petunidin 3-arabinoside	Pt 3-Ara	21.44	2.60
10	Peonidin 3-galactoside	Pn 3-Gal	20.68	1.36
11	Peonidin 3-glucoside	Pn 3-Glc	23.87	3.60
12	Malvidin 3-galactoside	Mv 3-Gal	23.87	3.60
13	Peonidin 3-arabinoside	Pn 3-Ara	26.43	0.61
14	Malvidin 3-glucoside	Mv 3-Glc	26.99	7.16
15	Malvidin 3-arabinoside	Mv 3-Ara	30.11	1.72

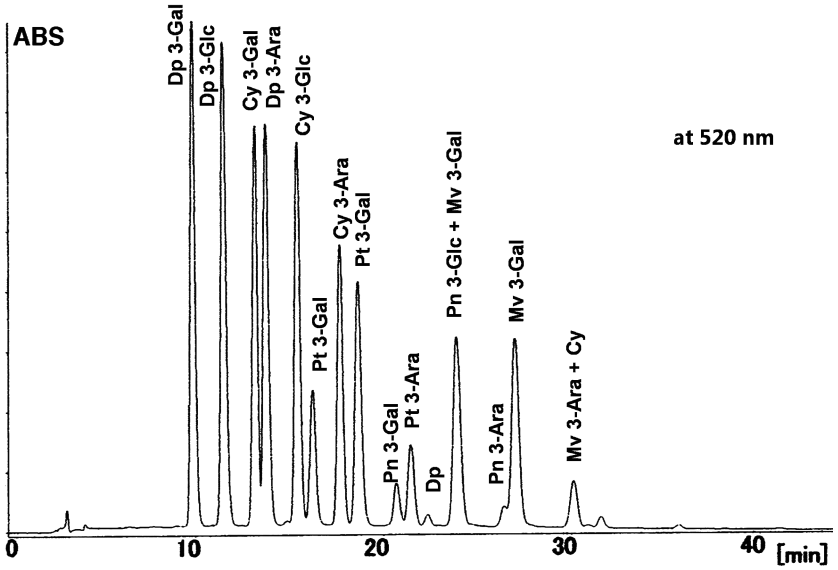

FIG. 1. High performance liquid chromatography (HPLC) profiling of anthocyanins in bilberry (*Vaccinium myrtillus*). The anthocyanin contents in the bilberry sample (Mirselect) were determined by HPLC (JASCO, Tokyo, Japan) using authentic anthocyanins as standards (See **Materials and Methods**). The upper table is a summary of the separated anthocyanins. The bottom diagram represents a typical pattern of HPLC profiling of anthocyanins.

reaction contained 250 ng of reversed transcripts, 75 ng of each primer, and 10 μl Master mix (Finnzymes Oy, Finland). The thermal cycling condition was held at 95°C for 15 min followed by 55 cycles of 30 s at 94°C, 30 s at Tm (Table 1) and 30 s at 72°C. The result was represented by relative expression level.

Statistical Analyses

Difference between treated and control cells were analyzed by Student's t-test. A probability of $P < 0.05$ was considered significant.

RESULTS

Gene Expression Profiles

Based on the results of our pilot experiments, RAW264.7 cells were treated with or without 75 μg/ml bilberry extracts for 30 min and then exposed to 40 ng/ml LPS for another 6 h. Cell mRNA was prepared and processed for hybridization to the mouse genome microarray as described in **Materials and Methods.** Comparing the signals of LPS treatment with those of the control revealed that a total of 3,084 gene signals were changed by ≥twofold, of which 998 gene signals were increased and 2,086 gene signals were decreased by ≥twofold (Table 2). Among the 3,084 genes responding to LPS, treatment

TABLE 1
Primers used for real-time PCR[a]

Gene	Sequence	Tm (°C)
COX-2	5′-gaa gtc ttt ggt ctg gtg cct g-3′	57
	5′-gtc tgc tgg ttt gga ata gtt gc-3′	57
IL-1β	5′-gag agc cgg gtg aca gta tc-3′	59
	5′-act tct gcc tga cga gct tc-3′	59
IL-6	5′-ctt cca tcc agt tgc ctt ct-3′	60
	5′-tcc acg att tcc cag aga ac-3′	60
PTGS2	5′-gct gta caa gca gtg gca aa-3′	64
	5′-ttc tgc agc cat ttc ctt ct-3′	64
TNC	5′-tgt gtg ctt cga agg cta tg-3′	60
	5′-gca gac aca ctc gtt ctc ca-3′	60
TNF	5′-gtg gaa ctg gca gaa gag gc-3	57
	5′-aga cag aag agc gtg gtg gc-3′	57
CCL22	5′-cat cat ggc tac cct gcg tgt ccc-3′	60
	5′-cct cct ccc tag gac agt tta tgg a-3′	60
IFI1	5′-aac agg ctc cag cag gtt ac-3′	59
	5′-gta agt ccc aca gca cca ca-3′	59
IFI47	5′-gct atc ctg gag gtg gct gt-3′	61
	5′-gcg tct gcg tgg aaa ttg-3′	61

[a]Abbreviations are as follows: PCR, polymerase chain reaction; Tm, temperature melting; COX-2, cyclooxygenase-2; IL, interleukin; PTGS, prostaglandin-endoperoxide synthase; TNC, tenascin C; TNF, tumor necrosis factor; CCL, CC chemokine ligand; IFI, interferon gamma inducible protein.

with bilberry extracts reduced 358 gene signals by ≥1.5-fold and enhanced 939 gene signals, respectively (Table 2). Therefore, bilberry extract affected approximately 42% of the LPS-responsive genes by ≥1.5-fold. This result suggests that bilberry might be effective in attenuating LPS-induced responses. To validate the results of microarray analysis, 6 representative genes with expression changes in several folds, several tens of folds, and several hundreds of folds by LPS were chosen, and their expression levels were detected by real-time PCR with the same RNA. The expression levels of those genes detected by real-time PCR exhibited a similar expression pattern with that of DNA microarray (data not shown), validating the use of DNA microarray data obtained in this study.

TABLE 2
The numbers of genes that are regulated by LPS and Bilberry extract[a]

Fold of Change	LPS/CTL	(B + LPS)/LPS
≤ 8	105	52
≤ 4 to <8	160	66
≤ 2 to <4	733	240
≤ 1 to <2	8,701	1,222
<–2 to ≤–1	10,269	1,358
<–4 to ≤–2	1,763	730
≤–4	323	209

[a]Abbreviations are as follows: LPS, lipopolysaccharide; CTL, control; B, bilberry.

Grouping of Genes Targeted by Bilberry

To understand the function of the target genes by bilberry extracts, 1,297 genes targeted by bilberry extracts (≥1.5-fold) were classified into the biological process, molecular function, and signaling pathway by the Panther gene group analysis software (http://www.pantherdb.org/). As shown in Table 3, 782 genes with ≥twofold change by LPS were classified into 43 groups. Of these, 309 genes were regulated by bilberry with ≥1.5-fold and classified into 36 groups hitting for biological processes (97), molecular functions (186), and signaling pathways (26) categories. The rest 10826 genes were unclassified. The gene groups highly affected by bilberry extracts were associated with "inflammatory response, signal transduction, cytokine activities, hydrolase activity, kinase activity, receptor activities, transferase activity, nucleic acid binding, and apoptosis" categories.

Profiling of Anti-Inflammatory Effects by Bilberry

To investigate the anti-inflammatory effects by bilberry, we next profiled the genes that are related to inflammatory response, defense, cytokine activity, and receptor activity. The changes of gene expression by bilberry with ≥1.5-fold are partially listed in Table 4. The induction of proinflammatory genes, including TNF, IL-1β, IL-6, TNC, PTGS2, and COX-2, by LPS were reduced by bilberry. In addition, the inductions of some CC and CXC chemokines including chemokine (C-C motif) ligand 17 (Ccl 17), 22 (Ccl 22), chemokine (C-X3-C motif) ligand 1 (Cx3cl 1), chemokine (C-X-C motif) ligand 11 (Cxcl 11), and 16 (Cxcl 16) by LPS were suppressed by bilberry. LPS-induced IFI1 and IFI 47 levels were also reduced by bilberry. The inductions of IL receptor (IL-10ra, IL23r, and IL4ra) by LPS were also attenuated by bilberry. On the other hand, LPS decreased the expression levels of a few CC chemokines (Ccl 11 and Ccl 25), IL-3, and receptors [IL-1ra12, IL-8ra, TNF receptor superfamily) 23 (Tnfrsf23), and Tnfsf4], whereas bilberry could restore them to normal level.

To confirm the results, the expression levels of IL-1β, IL-6 TNF, COX-1, PTGS2, TNC, CCL22, IFI47, and IFI1 were further detected by real-time PCR (Fig. 2). Most of them showed a similar expression pattern between microarray and real-time PCR data. For example, the level of LPS-stimulated expression of TNF was reduced by bilberry with 70% in the real-time PCR experiment, whereas the extent of reduction was 68% in the microarray experiment. The inhibitory effect of bilberry on IFI1 was 60% in the real-time PCR and 57% in the microarray experiments. IL-1β showed somewhat lower extent of bilberry's effect in the microarray (40%) than the extent in the real-time PCR (93%) experiment.

TABLE 3
Classification of genes targeted by bilberry in LPS-activated macrophages[a]

Category	CTL	LPS/CTL	(B + LPS)/CTL
Biological process			
1 Apoptosis	192	26	8
2 Carbohydrate metabolism	107	5	2
3 Cell adhesion	289	18	7
4 Cell cycle	247	8	4
5 Cell proliferation	69	11	4
6 Defense	121	13	6
7 Steroid metabolism	32	2	0
8 Protein metabolism and modification	16	1	0
9 Protein targeting	40	1	0
10 Protein localization	21	1	0
11 Signal transduction	546	53	27
12 Transport	1, 001	37	12
13 Lipid metabolic process	125	7	2
14 Inflammatory response	88	24	9
15 Cytokine activity	139	34	16
Molecular function			
1 Cell adhesion molecule activity	289	18	7
2 Cytoskeletal protein binding	25	1	1
3 Defense response	121	13	6
4 Cell adhesion	289	18	7
5 Hydrolase activity	978	51	18
6 Ion channel activity	233	8	4
7 Kinase activity	621	46	16
8 Ligase activity	134	5	1
9 Lyase activity	78	3	0
10 Protein ubiquitination	120	11	7
11 Nucleic acid binding	394	26	16
12 Oxidoreductase activity	384	18	6
13 Receptor activity	1, 512	84	35
14 Signal transduction	546	53	27
15 Transcription factor activity	500	34	9
16 Transferase activity	1, 015	67	22
17 Transporter activity	142	7	4
Pathway			
1 Angiogenesis	63	5	1
2 Apoptosis	192	26	8
3 B cell activation	8	4	2
4 Blood coagulation	42	4	0
5 Adhesion	289	18	7
6 EGF receptor signaling pathway	7	1	0
7 Cytokine and chemokine mediated signaling	21	6	2
8 Integrin-mediated signaling pathway	62	5	2
9 JAK-STAT cascade	11	4	1
10 Notch signaling pathway	38	2	1
11 Wnt receptor signaling pathway	81	3	2

[a] Abbreviations are as follows: LPS, lipopolysaccharide; CTL, control; B, bilberry; EGF, epidermal growth factor; JAK-STAT, janus activated kinase (JAK) signal transducers and activators of transcription (STAT); Wnt, wingless integration. LPS/CTL, >twofold; (B + LPS)/CTL, >1.5-fold.

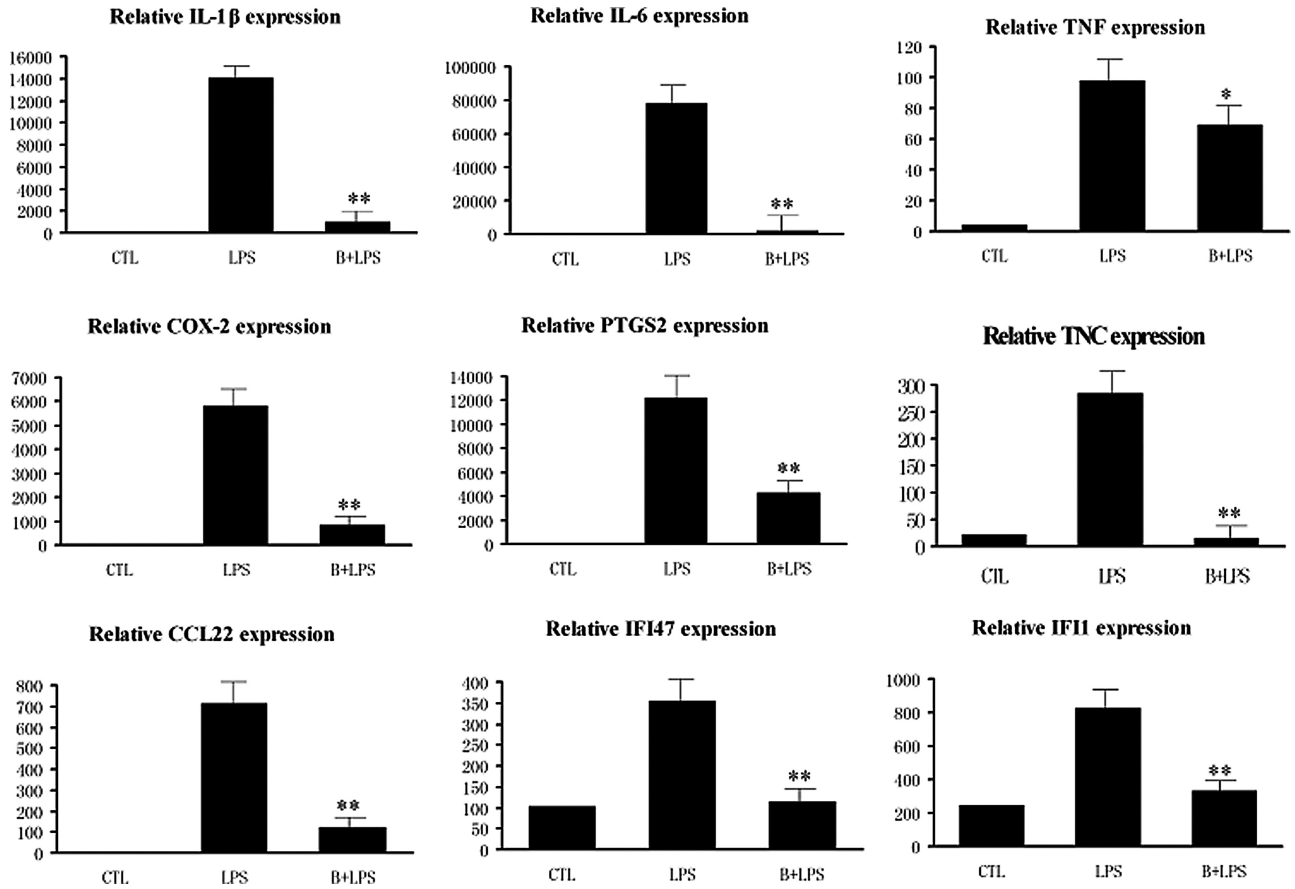

FIG. 2. Real-time polymerase chain reaction (PCR) of some genes identified by DNA microarray. RAW264.7 cells were pretreated with or without bilberry extracts for 30 min and then exposed to 40 ng/ml lipopolysaccharide (LPS) for 6 h. RNA extract and real-time PCR were described in **Material and Methods.** The primers used are shown in Table 1. The result was expressed by relative expression level. Each value represents mean± SD of 3 separate experiments. $*$, $P < 0.05$; $**$, $P < 0.001$ vs. LPS. IL, interleukin; TNF, tumor necrosis factor; COX-2, cyclooxygenase-2; PTGS2, prostaglandin-endoperoxide synthase 2; TNC, Tenascin C; CCL22, CC chemokine ligand 22; IFI, interferon-inducible protein.

DISCUSSION

Anthocyanins, anthocyanidins, or the crude extract from bilberry or other berries have been reported to attenuate COX-2 expression and/or PGE2 activity (9–11), suggesting anthocyanins may have anti-inflammatory activity through indeterminate molecular mechanisms. In this study, we, for the first time, showed the gene expression profiles of bilberry in RAW264 cell, a cell model system to study inflammation, by genome-wide DNA microarray.

Among a total of 22,050 gene probes, LPS treatment at 40 ng/ml for 6 h upregulated signals of 998 gene probes (4.5% of total gene probes) and downregulated signals of 2,086 gene probes (9.5% of total gene probes) by ≥twofold. These genes were categorized into 43 groups and hit for biological processes, molecular functions, and signaling pathways. The numbers of genes affected by LPS was fewer than that in the treatment with 1 μg/ml for 6 h reported previously (23), suggesting that RAW 264 cells responded to LPS in a dose-dependent manner. In our preliminary treatment, we found that high dose of LPS appeared cytotoxic to RAW246 cell; we thus treated the cells with 40 ng/ml LPS in this study to mimic inflammative response in a normal condition.

The number of genes affected by bilberry covered 45% of genes downregulated by LPS and 36% of genes upregulated by LPS. Analysis of the genes targeted by bilberry extracts revealed that a number of inflammation and defense-related genes were affected. The upregulation of proinflammatory genes, such as TNF, IL-1β, IL-6, TNC, PTGS, and COX-2 by LPS has been suggested to be a major factor for the inflammatory effects observed in LPS-activated macrophages (24, 25), and bilberry extracts appear to attenuate their expression. In addition, bilberry reduced the expression of IFI genes (IFI1 and IFI47). These factors are documented to be involved in IFN-mediated immunity and cell proliferation and differentiation (26). The inductions of IL receptor (IL-10ra, IL23r, and IL4ra) by LPS were also suppressed by bilberry. These results suggest that bilberry inhibition of the expression of some proinflammatory genes may explain its anti-inflammatory effects. On the other hand, bilberry

TABLE 4
List of genes targeted by bilberry in categories of defense, inflammatory response, cytokine activity, and receptor activity[a]

Gene	Accession No.	LPS/CTL	(B + LPS)/CTL
Blnk	NM_008528	2.29	1.52
Ccl17	NM_011332	17.64	13.50
Ccl22	NM_009137	6.79	0.60
Ccr2	NM_009915	2.52	0.69
Cd14	NM_009841	2.53	1.24
COX-2	AF378830	5.85	1.12
Cx3cl1	NM_009142	3.56	1.68
Cxcl11	NM_019494	2.20	0.87
Cxcl16	NM_023158	2.47	1.84
Ebi3	NM_015766	2.31	1.25
H2-T24	NM_008207	2.11	0.52
Ifi1	NM_008326	3.97	1.71
Ifi47	NM_008330	2.71	1.19
Il6	NM_031168	718.90	133.31
Il10ra	NM_008348	3.59	1.06
Il23r	NM_144548	2.34	0.99
Il4ra	NM_010557	2.74	1.90
Irak3	NM_028679	3.09	0.67
Lta	NM_010735	25.66	8.85
Ltb	NM_008518	2.34	0.43
Ltb4r1	NM_008519	2.53	1.62
Ptgs2	NM_011198	416.00	124.60
Ptger2	NM_008964	28.78	5.56
Tlr1	NM_030682	3.68	2.74
Tnf	NM_013693	2.49	1.70
Traf1	NM_009421	23.16	16.75
Tnfrsf5	NM_011611	6.81	3.78
Ccl11	NM_011330	0.24	1.74
Ccl25	NM_009138	0.28	0.91
Defb12	NM_152802	0.43	0.99
Hrh2	NM_008286	0.38	0.93
Ifna4	NM_010504	0.47	0.91
Il1rl2	NM_133193	0.48	0.85
Il3	NM_010556	0.22	0.92
Il8ra	NM_178241	0.36	0.69
Pigr	NM_011082	0.28	0.73
Ticam2	NM_173394	0.47	1.08
Tnfrsf23	NM_024290	0.33	0.76
Tnfsf4	NM_009452	0.22	0.75

[a]Abbreviations are as follows: LPS, lipopolysaccharide; CTL, control; B, bilberry.

could also restore the expression levels of LPS-reduced CC chemokines (Ccl 11 and Ccl 25), IL (IL-3), and receptors (IL-1ra12, IL-8ra, Tnfrsf23, and Tnfrsf4) to control levels. The data suggest that bilberry might not only attenuate the expression of some proinflammatory genes induced by LPS but also restore the expression level of anti-inflammatory genes reduced by LPS, although detailed information is required to obtain this in a further study.

Bilberry extract used in this study contained approximately 40% anthocyanins consisting of 15 varieties of anthocyanins glycosides (20). We have previously found that anthocyanins purified from bilberry exhibit a structure-activity relationship in the inhibition of COX-2 expression in LPS-activated macrophages (11). The orthodihydroxyphenyl structure on the B-ring of anthocyanidins appears to be important for the inhibitory action (11). Bilberry extracts contained approximately 80% of this type of anthocyanins including delphinidin glycosides, cyanidin glycosides, and petunidin glycosides (Fig. 1). Thus, anthocyanins in bilberry may constitute the major bioactive components responsible for the observed effects on gene expression as determined by microarray analysis. However, the effects of other components found in bilberry extract, such as some plant-derived polysaccharides, may also contribute to the observed changes.

In conclusion, our DNA microarray data for the first time provides gene expression profiling of bilberry extract in an inflammatory cell model, RAW264. Bilberry anthocyanins may target some proinflammatory genes to exert their potential function in anti-inflammation.

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