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Inhibition of Th2 Cytokine Production in T Cells by Monascin via PPAR-γ Activation

Wei-Hsuan Hsu,[†] Bao-Hong Lee,[†] Ya-Wen Hsu,[§] and Tzu-Ming Pan*[,†]

[†]Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan

[§]SunWay Biotechnology Company, Taipei, Taiwan

ABSTRACT: Yellow pigment monascin (MS) is a secondary metabolite isolated from *Monascus*-fermented products and has numerous physiological activities. However, the potential use of MS for immunomodulation remains unclear. We showed that MS and the synthetic peroxisome proliferator-activated receptor (PPAR)-γ ligand rosiglitazone (RG) significantly inhibited the production of Th2 cytokines, including IL-4, IL-5, and IL-13, in PMA/ionomycin-activated mouse EL-4 T cells. Moreover, we showed that this was due to cellular PPAR-γ translocation. These results indicate that MS and RG promote PPAR-γ−DNA interactions and suggest that the regulatory effects of MS and RG on Th2 cytokine production could be abolished with PPAR-γ antagonist treatment. MS and RG also suppressed Th2 transcription factor translocation (e.g., GATA-3 and nuclear factor of activated T cells) by preventing the phosphorylation of protein kinase C and signal transducer and activator of transcription 6.

KEYWORDS: *interleukin-4, monascin, Monascus-fermented metabolite, peroxisome proliferator-activated receptor (PPAR), T helper 2*

INTRODUCTION

When naive CD4 T cells encounter an antigen on antigen-presenting cells (APCs), they can develop into at least two distinct T helper (Th) type cells: (1) Th1 cells that secrete interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α; and (2) Th2 cells that secrete IL-4, IL-5, and IL-13.[1] Dysregulated expression of Th2 cytokine-producing cells has been linked to autoimmune and allergic diseases.[2] In T cells, Th2 cytokine gene expression is regulated at the transcriptional level by several nuclear transcription factors, such as GATA-3, nuclear factor of activated T cells (NF-AT), c-Maf, and signal transducer and activator of transcription 6 (STAT6).[3,4] These transcription factors interact with a proximal promoter region composed of multiple regulatory elements that can affect transcriptional activation. IL-4 gene transcription is mediated by subset-specific transcription factors such as GATA-3 and c-Maf during the differentiation of naive T cells into Th2 cells.[5]

Peroxisome proliferator-activated receptors (PPAR) are a family of ligand-activated nuclear receptor transcription factors. PPAR forms a heterodimer with the retinoic X receptor upon ligand binding. This complex then binds to PPAR-responsive elements (PPRE) located in the promoter regions of various genes and acts to regulate their expression.[6] Recently, much attention has been focused on PPAR-γ, because it also functions in adipogenesis and metabolism regulation as well as exerts a pleiotropic anti-inflammatory effect in organs. PPAR-γ is expressed in mouse T cells and has been reported to inhibit IL-4 production in both antigen-stimulated primary T cells and the phorbol 12-myristate 13-acetate (PMA)/ionomycin-activated T cell line to increase the number of regulatory CD4 T cells.[7,8]

Fermentation products of the fungus *Monascus*, especially those produced by solid-state fermentation, have been historically used as food colorants and dietary material. Rice fermented by *Monascus*, also known as red mold rice (RMR), is a common food and traditional medicinal remedy in Asian countries. Several chemical components of RMR have been purified and identified, including monacolins, γ-aminobutyric acid (GABA), pigments, and dimerumic acid. Yellow pigment monascin (MS) is a naturally occurring secondary metabolite with an azaphilonoid structure, isolated from *Monascus*-fermented products, and has cytotoxic and anti-inflammatory functions.[9−11] MS also reduces TNF-α-stimulated endothelial adhesiveness and down-regulates intracellular reactive oxygen species (ROS) formation, nuclear factor (NF)-κB activation, and vascular cell adhesion molecule-1 (VCAM-1) expression in human aortic endothelial cells.[12] Our research group has previously demonstrated that MS exhibits antiobesity properties through the regulation of adipogenesis and lipolysis.[13] In preadipocyte 3T3-L1 cells, MS inhibited cell proliferation and differentiation and decreased triglyceride accumulation.[13] MS also acts as a hypolipidemic and high-density lipoprotein cholesterol-raising agent.[14] We also showed that MS regulates endothelial adhesion molecules and endothelial NO synthase (eNOS) expression induced by TNF-α in human umbilical vein endothelial cells (HUVECs).[15] In C2C12 myotubes, MS improved TNF-α-induced insulin resistance by elevating PPAR-γ mRNA expression and attenuating PPAR-γ phosphorylation, thereby elevating the uptake of the deoxyglucose analogue 2-NBDG.[16]

Here, we studied the expression of PPAR-γ in mouse T cells and investigated the relationship between PPAR-γ activation and Th2 cytokine synthesis. We showed that MS and a synthetic PPAR-γ ligand, rosiglitazone (RG), significantly

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inhibited Th2 cytokine production in a phorbol 12-myristate 13-acetate (PMA)/ionomycin-activated T cell line. Our results show that PPAR-γ ligand-mediated suppression of Th2 cytokine production from activated T cells may involve, at least in part, both the promotion of PPAR-γ–DNA interactions and the inhibition of protein kinase C (PKC) phosphorylation.

MATERIALS AND METHODS

Chemicals and Reagents. PMA, RG, PPAR-α agonist WY14643 (WY), and trypsin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Life Technologies (Carlsbad, CA, USA). Dimethyl sulfoxide (DMSO) was purchased from Wako Pure Chemical Industries (Saitama, Japan). Roswell Park Memorial Institute (RPMI)-1640 medium was purchased from HyClone Laboratories (Logan, UT, USA). Sodium dodecyl sulfate (SDS) was purchased from Merck (Darmstadt, Germany). The Bio-Rad protein assay dye was from Bio-Rad Laboratories (Hercules, CA, USA). PPAR-α antagonist MK886 (MK) and PPAR-γ antagonist GW9662 (GW) were purchased from Cayman Chemical (Ann Arbor, MI, USA).

Monascin Isolation and Purification. Monascin (Figure 1A) was isolated from *Monascus*-fermented rice (red mold rice) by SunWay Biotechnology Co. (Taipei, Taiwan). Briefly, the crude extracts of *Monascus*-fermented rice were obtained after filtering and concentrating under reduced pressure and then coated on silica gel and subjected to dry flash chromatography. Sufficient *n*-hexane was passed through the column to remove the oily hydrophobic materials. Extensive gradient elution was then employed using different ethyl acetate in *n*-hexane ratios to yield numerous fractions. Similar fractions were combined according to thin layer chromatography, and the solvent was removed under reduced pressure. These fractions were further analyzed by high-performance liquid chromatography, and then fractions with a similar single peak profile were combined, respectively. Finally, the fraction with the desired compound was concentrated to dryness. Preparation of MS (>95% purity) was identified according to our previous study by nuclear magnetic resonance (NMR, Varian Gemini, 200 MHz, FT-NMR, Varian Inc., Palo Alto, CA, USA) and electrospray ionization–mass spectrometry (ESI-MS, Thermo Electron Co., Waltham, MA, USA) analysis.[17]

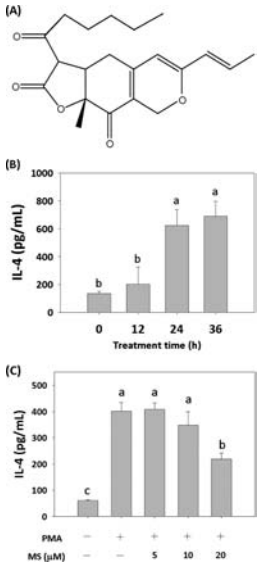

Figure 1. MS inhibited IL-4 secretion from PMA/ionomycin-activated EL-4 T cells: (A) chemical structure of MS; (B) IL-4 production in the medium of EL-4 cells after PMA (10 μg/mL) and ionomycin (400 nM) treatment for 12, 24, and 36 h; (C) effect of MS on IL-4 production after 24 h of treatment. MS (20 μM) significantly decreased IL-4 levels in PMA/ionomycin-activated T cells. Results are expressed as the mean ± SD (n = 3). Different letters above bars indicate significant difference from each other (p < 0.05). PMA, phorbol 12-myristate 13-acetate; MS, monascin.

Cell Culture. EL-4 murine T-lymphoma cells were purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan). EL-4 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS. Confluent cells were subcultured at a ratio of 1:3 in a 10 cm dish, and media were changed twice a week. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2. Th2 cytokine production in EL-4 T cells were determined after PMA (10 μg/mL) and ionomycin (400 nM) treating with or without MS (20 μM) for 24 h.

Quantification of Cytokines by ELISA. After 24 h of incubation, the culture medium of control and treated cells was collected, centrifuged, and stored at −80 °C until tested. The levels of IL-4, IL-5, and IL-13 released into culture medium were determined with ELISA kits from eBioscience (San Diego, CA, USA).

Nuclear and Cytosol Protein Extraction. After 0, 4, 8, 12, or 24 h of treatment, cells were collected for nuclear protein extraction according to the fractionation kit protocol supplied from BioVision (Mountain View, CA, USA). In brief, harvested cells were added to 200 μL of cytosol extraction buffer containing dithiothreitol (DTT) and protease inhibitors. After vortexing and 10 min of incubation on ice, the extracts were centrifuged at 16000g for 5 min at 4 °C, and the supernatant was removed to separate the cytoplasmic fraction from nuclei. The nuclei pellets were then added to 100 μL of nuclei extraction buffer, vortexed briefly, and set on ice every 10 min for total 40 min. After centrifuging at 16000g for 10 min, the supernatant nuclear extracts were stored at −80 °C for future use. The protein concentration in the cell extract was determined using a Bio-Rad protein assay kit.

Immunoblot Analysis. The samples were subjected to 10% SDS–polyacrylamide gel electrophoresis (PAGE). The protein spots were electrotransferred to a polyvinyldiene difluoride (PVDF) membrane. The membrane was incubated with block buffer (phosphate-buffered saline (PBS) containing 0.05% Tween-20 and 5% w/v nonfat dry milk) for 1 h, washed with PBS containing 0.05% Tween-20 (PBST) three times, and then probed with 1:1000 diluted solution of anti-GATA-3, anti-NF-ATc, anti-T-bet, anti-c-Maf, anti-STAT6, and anti-p-STAT6 antibodies (Abcam, Cambridge, MA, USA) and anti-PPAR-γ (1:2000), anti-PKC (1:1000), and anti-p-PKC (1:1000) antibodies (Cell Signaling Technology, Beverly, MA, USA) overnight at 4 °C. In addition, the intensity of the blots probed with 1:1000 diluted solution of mouse monoclonal antibody to bind GAPDH (Cell Signaling Technology) or Lamin B (Santa Cruz Biotechnology Inc., Burlingame, CA, USA) was used as the control to ensure that a constant amount of protein was loaded into each lane of the gel. The membrane was washed three times each for 5 min in PBST, shaken in a solution of horseradish peroxidase (HRP)-linked anti-rabbit IgG secondary antibody, and washed three more times each for 5 min in PBST. The expressions of proteins were detected by enhanced chemiluminescent (ECL) reagent (Millipore, Billerica, MA, USA) and image reader (LAS-3000, Fuji, Tokyo, Japan).

DNA Binding Activity of PPAR-γ. After treatment with samples for 6, 12, and 24 h, nuclear extracts were prepared from EL-4 T cells by means of a commercially available transcription factor ELISA kit (Trans-Binding PPAR-γ assay) (Panomics, Redwood City, CA, USA) and performed according to the instructions of the manufacturer. Briefly, oligonucleotides encoding for the PPAR-γ consensus binding site were bound to microplates. Subsequently, the transcriptional activity of activated PPAR-γ in the prepared nuclear extracts was determined by PPAR-γ binding to the immobilized DNA and confirmed with PPAR-γ antibody using ELISA technology.

Statistical Analysis. The statistical significance was determined by one-way analysis of variance (ANOVA) using the general linear model procedure of SPSS software (SPSS Institute, Inc., Chicago, IL, USA), followed by ANOVA with Duncan's test. The results were considered to be statistically significant if the p value was <0.05. Data are expressed as the mean ± SD.

RESULTS

MS Mediated Down-regulation of T Cells Th2 Cytokine Secretion. We first tested the effect of PMA/ionomycin on T cell activation. IL-4 production was detected by examining the media of EL-4 T cells after PMA (10 μg/mL) and ionomycin (400 nM) treatment for 12, 24, and 36 h. After induction for 24 h, IL-4 secretion from EL-4 cells was significantly enhanced when compared with the control group (Figure 1B). Next, EL-4 cells were cotreated for 24 h with PMA/ionomycin and different concentrations of MS. The data indicate that 20 μM MS treatment markedly inhibited IL-4 synthesis (Figure 1C), which suggests that MS might potentially act to block Th2 signals in T cells. As such, 20 μM MS was chosen for future experiments.

To investigate the effects of PPAR on Th2 cytokine production, we determined IL-4, IL-5, and IL-13 levels by treatment with MS, a PPAR-γ agonist RG, a PPAR-α agonist WY, and/or PMA/ionomycin and tested the media of EL-4 cells after 24 h. Our data indicate that MS significantly reduced IL4, IL-5, and IL-13 levels in the medium of PMA/ionomycin-activated EL-4 cells, whereas MS treatment alone did not influence the release of Th2 cytokines when compared to the control group (Figure 2). The PPAR-γ agonist RG and the PPAR-α agonist WY also exhibited similar effects in response to MS treatment (Figure 2). However, the inhibitory effect of MS on Th2 cytokine levels was abated with the addition of GW, a PPAR-γ antagonist (Figure 2). This finding suggests that the effect of MS on T cell Th2 cytokine secretion was partly mediated by PPAR-γ. In contrast, treatment of EL-4 cells with the PPAR-α antagonist MK had no effect on MS-mediated inhibition of Th2 cytokine production (Figure 2).

MS Elevated PPAR-γ Translocation in T Cells. Figure 3A shows no significant difference in the amount of total PPAR-γ protein for each group. We next extracted nuclear and cytosolic proteins and examined PPAR-γ expression after MS or RG treatment after 0, 4, 8, and 12 h. After treatment with 20 μM MS for 8 h, the translocation of PPAR-γ from the cytoplasm to the nucleus was increased, with the most prominent translocation occurring at 8 and 12 h after treatment. A similar pattern was observed in RG-treated T cells (Figure 3B). We next examined the effect of MS on the DNA-binding activity of PPAR-γ. As shown in Figure 4, treatment with MS or RG significantly elevated PPAR-γ binding to DNA at 12 and 24 h after treatment, whereas PMA/ionomycin slightly attenuated this binding activity after 24 h of treatment. Moreover, MS and RG significantly restored the reduction of PPAR-γ-binding activity caused by PMA/ionomycin treatment at 12 and 24 h. This finding suggests that PPAR-γ may be involved in suppressing MS-mediated PMA/ionomycin-induced T cell activation.

MS Regulated Th2 Signal Transduction. We next determined if there exists a relationship between the MS-induced inhibition of Th2 signals and its effect on PPAR-γ expression. Our results indicate that the expression of T-bet, a Th1-specific T box transcription factor that controls the expression of the hallmark Th1 cytokine, IFN-γ, was similar in each group (Figure 5A,B). In contrast, expression of the Th2 transcription factors GATA-3, NF-AT, and c-Maf were markedly increased in PMA/ionomycin-activated T cells (Figure 5A,C–E). We found that MS and RG reduced the levels of GATA-3 and NF-AT, especially the levels of GATA-3. However, inhibition of PPAR-γ with the PPAR-γ antagonist

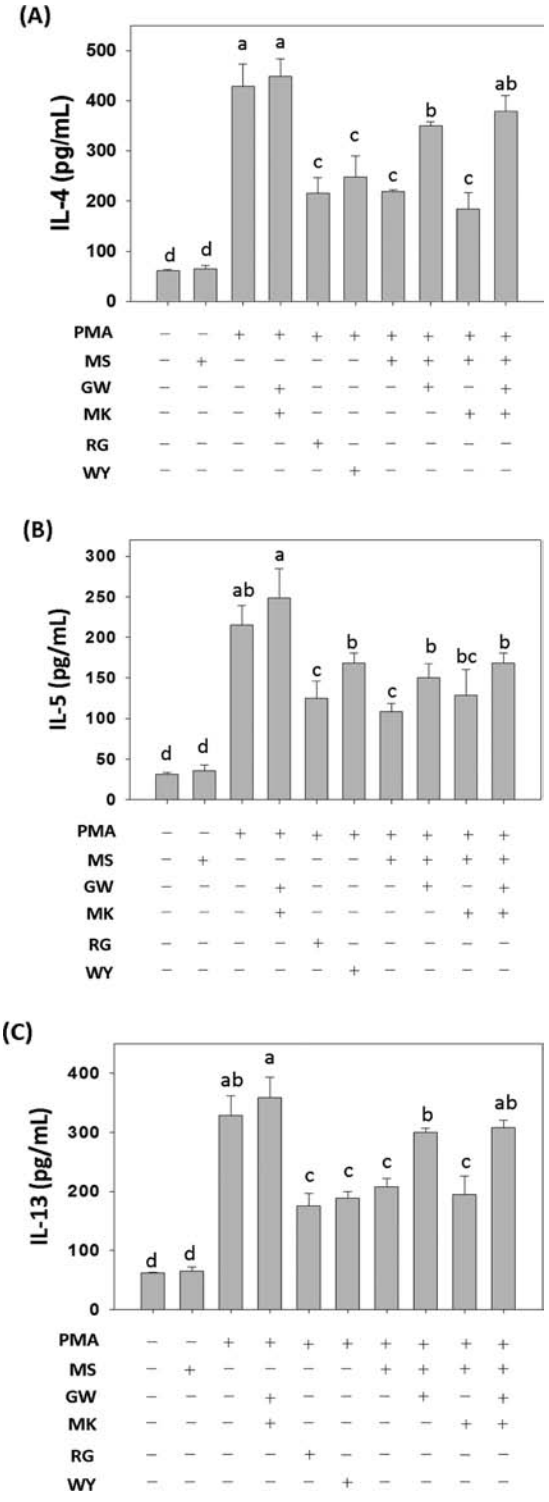

Figure 2. MS-mediated down-regulation of T cells Th2 cytokine secretion. MS markedly reduced (A) IL4, (B) IL-5, and (C) IL-13 levels in the medium of PMA/ionomycin-activated EL-4 cells after 24 h of treatment. RG and WY also exhibited similar effects in response to MS treatment. The inhibitory effect of MS on Th2 cytokine levels was abated with the addition of GW. Results are expressed as the mean ± SD (n = 3). Different letters above the bars indicate significant difference from each other ($p < 0.05$). PMA, phorbol 12-myristate 13-acetate; MS, monascin; GW, PPAR-γ antagonist GW9662; MK, PPAR-α antagonist MK886; RG, PPAR-γ agonist rosiglitazone; WY, PPAR-α agonist WY14643.

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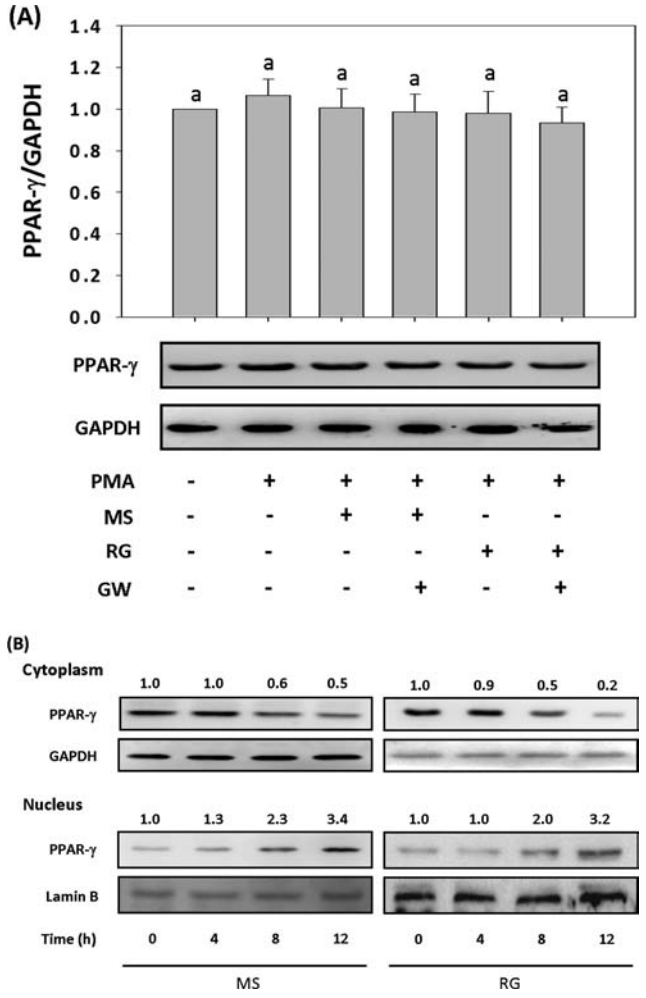

Figure 3. MS-promoted PPAR-γ translocation from cytoplasm into nucleus: (A) no significant differences in the amount of total PPAR-γ protein for each group; (B) PPAR-γ expression in nuclear and cytosolic proteins in T cells after MS or RG treatment after 0, 4, 8, and 12 h. The most prominent translocation by MS occurred at 8 and 12 h after treatment. Results are expressed as the mean ± SD (n = 3). PMA, phorbol 12-myristate 13-acetate; MS, monascin; RG, PPAR-γ agonist rosiglitazone; GW, PPAR-γ antagonist GW9662.

GW did not affect MS and RG-mediated Th2 transcription factor expression (Figure 5A,C,D).

We further investigated Th2 signals in PMA/ionomycin-activated EL-4 cells. Our data suggest that MS and RG significantly suppressed PMA/ionomycin-induced STAT6 and PKC phosphorylation, which is required for responsiveness to IL-4 and IL-13 (Figure 6). We found that GW did not abolish p-STAT6 and p-PKC inhibition in the MS- and RG-treated groups (Figure 6), which means that there may exist some other pathway involved in the MS-regulated T cell inactivation.

MS Regulated Th2 Signals in a PPAR-γ-Dependent Pathway. To understand whether MS-regulated Th2 cytokine expression was associated with PPAR-γ, we treated EL-4 cells with PMA/ionomycin for 12 h to activate PKC and then added MS or RG for another 12 h. As shown in Figure 7, in EL-4 cells, MS and RG retained the ability to decrease IL-4 production, throughout MS- and RG-mediated inhibition of IL-4 secretion. This effect was weaker than the effect observed earlier, as shown in Figure 2A.

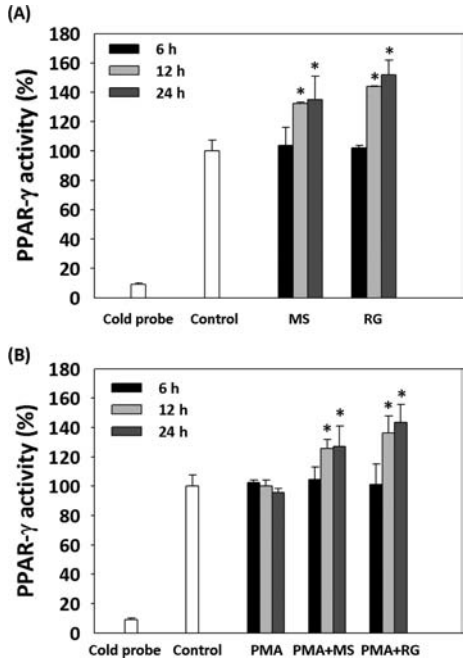

Figure 4. Effect of MS on the DNA binding activity of PPAR-γ. (A) MS and RG clearly elevated PPAR-γ binding to DNA for 12 and 24 h of treatment. (B) PMA/ionomycin slightly decreased DNA binding activity compared to the control group after 24 h of treatment. MS and RG significantly increased PPAR-γ binding activity in PMA/ionomycin-activated T cells at 12 and 24 h. Results are expressed as the mean ± SD (n = 3). An asterisk (*) indicates significant difference from the control group at $p < 0.05$. PMA, phorbol 12-myristate 13-acetate; MS, monascin; RG, PPAR-γ agonist rosiglitazone.

DISCUSSION

Monascus-fermented products have been used as traditional food in Asia for several centuries. The yellow pigment MS derived from *Monascus* is a secondary metabolite with an azaphilonoid structure and has been reported to exhibit cytotoxic,[9] immunosuppressive,[10] and anti-inflammatory activities.[11] Our group previously demonstrated that MS exerts antiobesity effects through adipogenesis and lipolysis regulation.[13] In preadipocyte 3T3-L1 cells, MS inhibited cell proliferation and differentiation and decreased triglyceride accumulation by suppressing the expression of transcription factors such as C/EBPs and PPAR-γ.[13] This effect occurred because PPAR-γ is responsible for regulating adipocyte differentiation, and insulin stimulation enhances PPAR-γ expression to induce cell differentiation.[18] MS also acts as a hypolipidemic and high-density lipoprotein cholesterol-raising agent.[14] In HUVECs, MS stimulates endothelial NO synthase (eNOS) expression and attenuates adhesion-factor expression induced by TNF-α.[15] Moreover, we found that MS can exhibit antidiabetic activity; it increases PPAR-γ mRNA expression and prevents PPAR-γ phosphorylation, thereby elevating uptake of the deoxyglucose analogue 2-NBDG in C2C12 myotubes.[16] However, whether MS has the potential to regulate immune responses remains unclear.

In this study, we demonstrated that both MS and the PPAR-γ ligand RG inhibited Th2 cytokine production in the PMA/ionomycin-activated EL-4 T cells. This inhibitory effect was due to, at least in part, the down-regulation of PKC and STAT6 phosphorylation and promotion of PPAR-γ translocation via binding to the PPAR-γ sites (Figure 8). This finding, specifically

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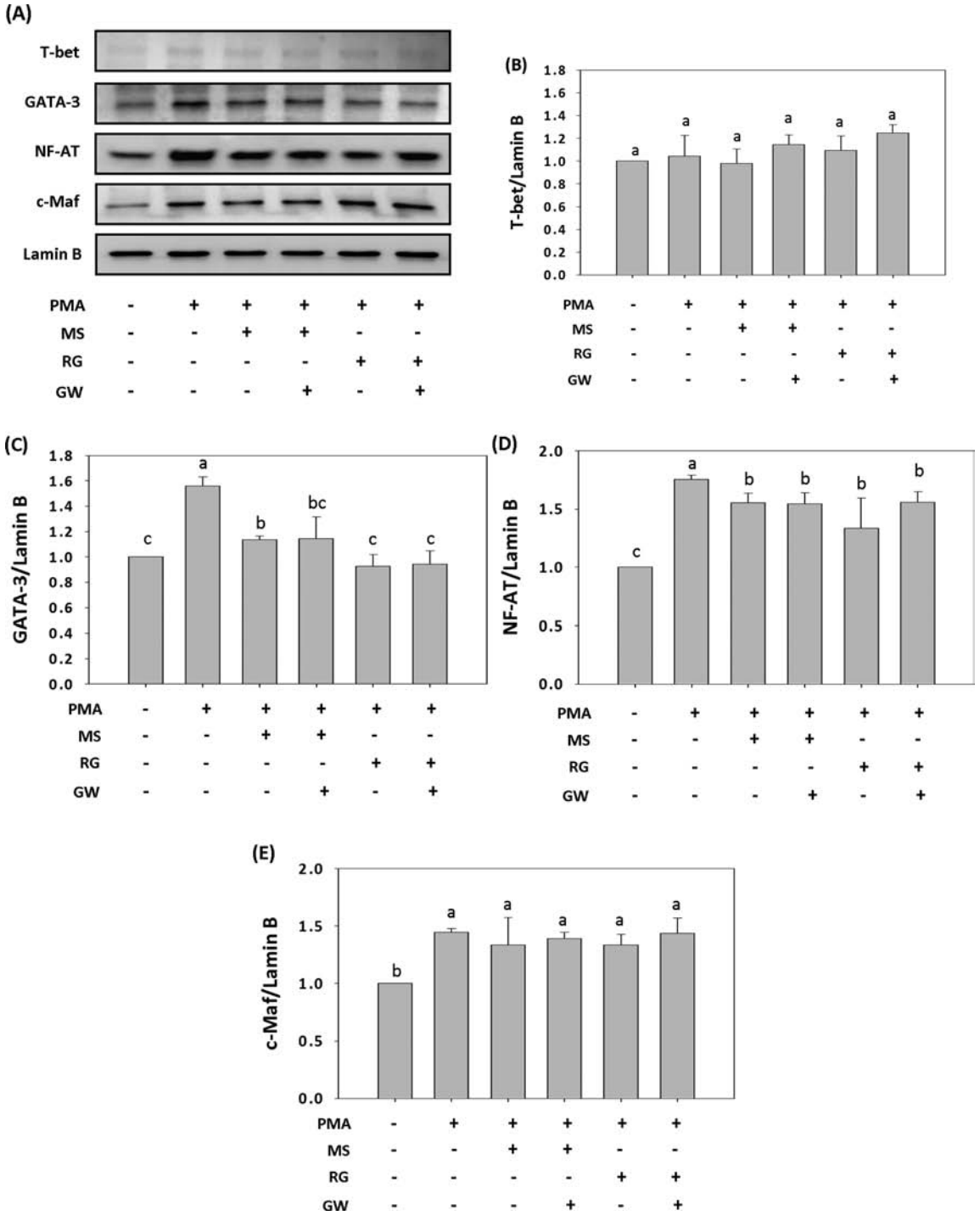

Figure 5. Regulation of Th transcription factor expression by MS in EL-4 T cells. (A) Western blot analysis of nuclear protein extracts obtained from EL-4 cells treated with PMA/ionomycin and MS for 24 h. Densitometric analysis of (B) T-bet, (C) GATA-3, (D) NF-AT, and (E) c-Maf levels. MS and RG reduced GATA-3 and NF-AT levels, and inhibition of PPAR-γ with GW did not affect MS and RG-mediated Th2 transcription factor expression. Results are expressed as the mean ± SD (n = 3). Different letters above bars indicate significant difference from each other ($p < 0.05$). PMA, phorbol 12-myristate 13-acetate; MS, monascin; RG, PPAR-γ agonist rosiglitazone; GW, PPAR-γ antagonist GW9662.

that MS and PPAR-γ ligands affect the synthesis of Th2 cytokine via T cells, is of special interest because the Th2 cytokines IL-4 and IL-13 mediate important pro-inflammatory functions in asthma. This includes the induction of IgE isotype switching, expression of VCAM-1, acceleration of eosinophil transmigration across the endothelium, and promotion of Th2-mediated immune responses.[19−21]

PPAR-γ and its ligands are also implicated in the pathology and/or treatment of numerous immune- and inflammation-related diseases.[22] PPAR-γ agonists, including 15d-PGJ_2, ciglitazone, and troglitazone, have been shown to significantly inhibit T cell proliferation; this is accompanied by a decrease in cell viability and induction of apoptosis.[23] The concept of PPAR-γ-induced apoptosis of T cells raises the possibility that PPAR-γ agonists in T cell-related diseases might be used as

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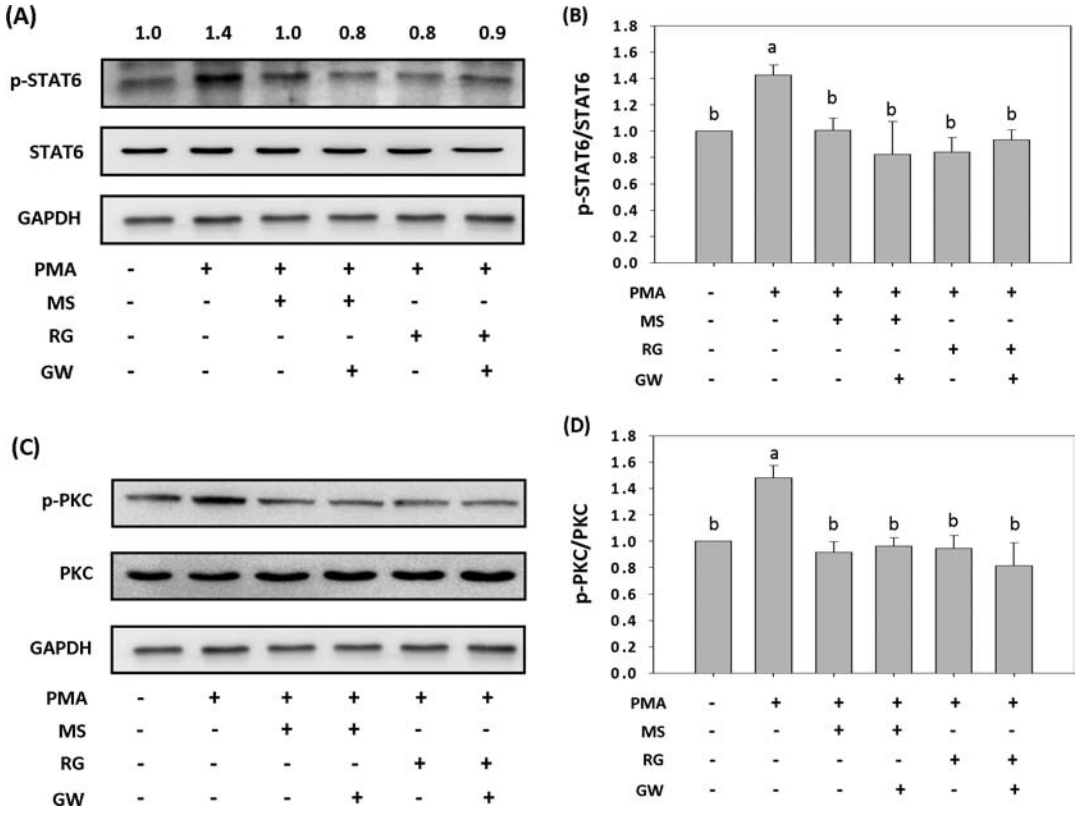

Figure 6. Modulation of MS on Th2 signal transduction. After incubation for 6 h, cells were lysed and immunoblotted with antibodies against (A) phosphorylated-STAT6 and (C) phosphorylated-PKC. Each protein was normalized with total STAT6 and PKC. Densitometric analysis of (B) p-STAT6/STAT6 and (D) p-PKC/PKC levels. MS and RG significantly suppressed PMA/ionomycin-induced STAT6 and PKC activation, whereas GW did not abolish p-STAT6 and p-PKC inhibition in the MS- and RG-treated groups. Results are expressed as the mean ± SD (n = 3). Different letters above bars indicate significant difference from each other (p < 0.05). PMA, phorbol 12-myristate 13-acetate; MS, monascin; RG, PPAR-γ agonist rosiglitazone; GW, PPAR-γ antagonist GW9662.

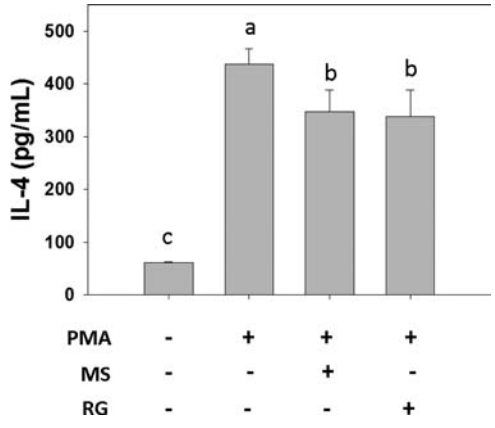

Figure 7. MS regulated Th2 signals in a PPAR-γ-dependent pathway. EL-4 cells were treated with PMA/ionomycin for 12 h and then added MS or RG for another 12 h. MS and RG retained the ability to decrease IL-4 production, throughout MS and RG-mediated inhibition of IL-4 secretion. Results are expressed as the mean ± SD (n = 3). Different letters above bars indicate significant difference from each other (p < 0.05). PMA, phorbol 12-myristate 13-acetate; MS, monascin; RG, PPAR-γ agonist rosiglitazone.

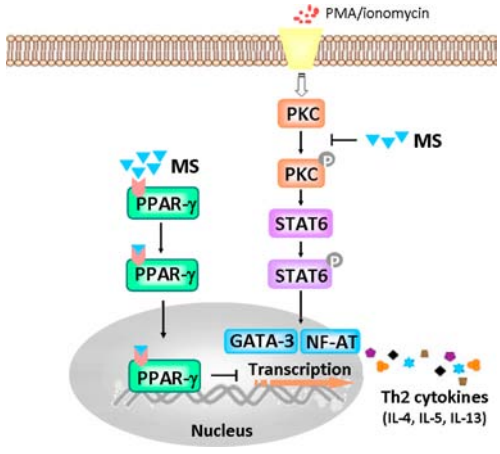

Figure 8. Potential mechanism of monascin (MS) in inhibiting Th2 cytokine production in EL-4 T cells mediated by PPAR-γ.

therapeutics. Interestingly, it has been found that at concentrations required to induce transcriptional activation, thiazolidinedione (TZD) activation of PPAR-γ protects T cells from apoptosis.[24] In murine and/or human systems, PPAR-γ ligands inhibit the production of additional proinflammatory cytokines such as IFN-γ and TNF-α.[25−27] Other PPAR-γ-independent effects of 15d-PGJ_2 were also reported: induction of expression of the proinflammatory cytokine IL-8 in stimulated human T cells[28] or enhancement of expression of heme oxygenase-1, an anti-inflammatory enzyme, in human lymphocytes.[29]

The expression of T cell-specific transcription factors and the PKC pathway are known to play critical roles in T cell activation and to affect the gene expression of Th2

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cytokines.[30,31] Several transcription factors critical for IL-4 transcription, including STAT6,[32] NF-AT,[33] c-Maf,[34] and GATA3,[35] have since been identified. GATA-3 belongs to the GATA family of transcription factors, which is an important regulator of T cell development and also plays a role in endothelial cell biology. GATA-3 promotes the secretion of IL-4, IL-5, and IL-13 from Th2 cells.[36] NF-AT is a general name for a family of transcription factors that aid in the immune response. One or more members of the NF-AT family are expressed in most cells of the immune system, and this transcription factor promotes cytokine expression and T cell activation.[37] STAT6 is essential for mediating responses to IL-4 lymphocytes. PMA/ionomycin treatment induces PKC phosphorylation in T cells, which further activates STAT6. Moreover, STAT6-deficient T lymphocytes fail to differentiate into Th2 cells in response to either IL-4 or IL-13.[32] In this study, we found that both MS and RG have the ability to inhibit the Th2 transcription factors GATA-3 and NF-AT translocation into the nucleus and therefore block Th2 signals (e.g., p-PKC and p-STAT6). Surprisingly, treatment with GW, the PPAR-γ antagonist, did not abrogate MS and RG-mediated inhibitory effects. This finding suggests that MS and RG might regulate Th2 cytokine production both in a PPAR-γ-dependent pathway and by directly inhibiting PKC activation via an unknown mechanism. Some studies have demonstrated that PPAR-γ agonists have been found to suppress PKC activation in different cell models.[38,39] Due to this development, we modified the experiment model and treated EL-4 cells with PMA/ionomycin first for 12 h to induce PKC phosphorylation and then treated with MS or RG for another 12 h. Our data indicate that MS and RG might decrease IL-4 production, thus demonstrating that MS and RG could regulate IL-4 secretion through PPAR-γ.

In summary, the results of the current study indicate that the natural PPAR-γ activator, MS, and the synthetic PPAR-γ agonist, TZD, mediate the suppression of Th2 cytokine production by activated T cells. This process likely involves both the promotion of PPAR-γ−DNA interactions and the inhibition of PKC activation. Further investigation concerning the effect of MS on the regulation of immune responses is needed to better understand any potential therapeutic mechanisms.

AUTHOR INFORMATION

Corresponding Author

*(T.-M.P.) Phone: +886-2-33664519, ext. 10. Fax: +886-2-33663838. E-mail: tmpan@ntu.edu.tw.

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Notes

The authors declare no competing financial interest.

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