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Preferential induction of Th17 cells *in vitro* and *in vivo* by Fucogalactan from *Ganoderma lucidum* (Reishi)

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ABSTRACT

The mushroom known as Reishi (*Ganoderma lucidum*) has been used as an herbal medicine for tumor treatment and immune system activation. Because its effects on the differentiation of effector T helper cells have not yet been fully understood, we investigated the effects of Reishi and those of its principal ingredient, β-glucan, on the activation of dendritic cells and the differentiation of Th17 cells. Reishi extracts as well as purified β-glucan (Curdran) activated DCs and caused them to produce large amounts of IL-23. β-glucan also enhanced and sustained the transcription of IL-23p19. The MEK-ERK signaling pathway positively regulates IL-23p19 transcription in β-glucan-stimulated DCs. In a mixed leukocyte reaction, Reishi-stimulated DCs preferentially induced Th17 cells. Furthermore, orally-administrated Reishi increased the percentages of Th17 cells and the transcription levels of antimicrobial peptides. Our results show that Reishi and β-glucan activate DCs to produce large amounts of IL-23, which induces Th17 differentiation both *in vitro* and *in vivo*.

1. Introduction

Reishi (*Ganoderma lucidum*), a popular home remedy in Asia, has been known for centuries to have beneficial effects on human health. It has been reported to stimulate immune function, thereby inhibiting tumor growth and ameliorating chronic hepatitis, hypertension, and hyperglycemia [1]. It has proposed that this activation of the immune system occurs through certain effects on antigen-presenting cells (APC) and mononuclear phagocytes [2]. Despite numerous studies on the subject, however, the precise mechanism by which Reishi affects the differentiation of effector T helper cells has remained unclear.

The differentiation of naïve $CD4^+$ T cells into effector T helper cells is initiated by the engagement of their T cell receptors (TCR) and costimulatory molecules in the specific cytokines produced by the innate immune system. IFNγ and IL-12 initiate the differentiation of Th1 cells, which promote immunity to viruses, intracellular bacteria and protozoan parasites. In contrast, IL-4 triggers the differentiation of Th2 cells, which play a key role in organizing host defense against metazoan parasites and in helping B cells to produce antibodies. Recently, a third subset of effector Th cells was discovered: Th17 cells, which produce IL-17 and also exhibit other effector functions distinct from those of Th1 and Th2 cells [3,4]. IL-17 and/or its receptor have been linked to resistance to infection by extracellular bacteria such as *Klebsiella pneumoniae* as well as by fungi such as *Candida albicans* [5,6]. Indeed, Th17 cells develop during infection with *C. albicans*, and now believed to be important for antifungal immunity [7].

IL-6 and TGF-β are essential for Th17 cell differentiation [3,4]. In addition, the expansion and full differentiation of Th17 cells is thought to require IL-23, a heterodimer composed of a p19 subunit and the p40 subunit shared with IL-12 [8,9]. Recently, C-type lectins, especially Dectin-1 and Dectin-2 on dendritic cells have been implicated in the induction of anti-fungal infection by inducing IL-23 and Th17 [10–12]. However, precise mechanism of IL-23 expression by C-type lectins remained to be investigated.

To date, little is known about the effects of Reishi on Th17 cell differentiation. Here, we investigated the effects of Reishi on murine immune cells, especially DCs and T helper cells. We found that Reishi can enhance Th17 differentiation both *in vitro* and *in vivo* through enhancing the production of IL-23 by DCs. We also found that the activation of the MEK-ERK pathway is important in the induction of IL-23 by Reishi. Various immuno-modulatory effects of Reishi reported previously could be partly accounted by induction of Th17 in the gut.

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2. Materials and methods

2.1. Mice and reagents

C57BL/6 mice obtained from Charles River (Wilmington, MA, USA) were used for the experiments. Mice were maintained under specific pathogen-free conditions and offered food and water *ad libitum*. All experiments were approved by the Animal Ethics Committee of Kyushu University. Reishi and water-soluble fraction of Reishi were kindly provided by Nissan Chemical (Tokyo, Japan). Curdlan was purchased from Wako (Osaka, Japan). PGN was purchased from Fluka (Buchs, Switzerland). LPS was purchased from Sigma–Aldrich (St. Louis, MO, USA). U0126 was purchased from Calbiochem (San Diego, CA, USA).

2.2. BMDC preparation

BMDCs were prepared from bone marrow suspension obtained from the femurs and tibias of C57BL/6 mice, as described elsewhere [13]. Bone marrow cells were cultured in RPMI1640 medium (Sigma–Aldrich) supplemented with 10% FCS (Biological Industries, Beit Haemek, Israel), L-glutamine, β-mercaptoethanol, Penicillin–Streptomycin and culture supernatant from J558L cells transfected with the murine GM-CSF gene. After 8 days, cells were harvested, counted, and then used as BMDCs.

2.3. Surface and intracellular cytokine staining

Surface markers of DCs were stained with anti-CD11c-APC, anti-CD86-PE and anti-CD40-FITC (eBioscience, San Diego, CA, USA) after Fc receptor blocking using anti-mouse CD16/32 MAb. Tissue T cells were stained with anti-CD3ε-FITC or APC (eBioscience), anti-CD4-PerCP (BD Bioscience, Franklin Lakes, NJ, USA). Intracellular Cytokine Staining was performed as described elsewhere [14]; briefly, cultured cells were restimulated for 6 h with 50 nM PMA (Sigma–Aldrich), 1 μg/ml ionomycin (Sigma–Aldrich), and 1 μM brefeldin A (eBioscience). Surface staining was performed for 15 min with the corresponding mixture of fluorescently labeled Abs. After surface staining, the cells were suspended in Fixation Buffer (eBioscience), and intracellular cytokine staining was performed as per the manufacturer's protocol using anti-IFN-γ-FITC, anti-Foxp3-APC (eBioscience), and anti-IL-17-PE (BD Bioscience).

2.4. Western blotting analysis

Western blotting was performed as described elsewhere [13]. Briefly, BMDCs (5×10^5) were treated with LPS (100 ng/ml), Curdlan (1 mg/ml) or Reishi (1 mg/ml) for the lengths of time indicated, and then collected and lysed with NP40 lysis buffer. Proteins were separated by 8% SDS–PAGE, transferred onto nitrocellulose membranes and incubated with antibodies reactive to anti-phospho-ERK(P-p44/p42 MAPK), anti-phospho-IκB, anti-phospho-JNK (Cell Signaling Technology, Danvers, MA, USA) or total-ERK2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.5. Real time-PCR

Real time-PCR was performed as described elsewhere [16]. Briefly, total RNA was extracted using the RNA-iso (Takara Bio, Shiga, Japan) according to the manufacturer's protocol, and cDNA was then synthesized with the High Capacity cDNA reverse transcription kit (Applied BioSystems, Carlsbad, CA, USA). The cDNA was used as a template for real time PCR using the SYBR Green system (Applied BioSystems) according to the manufacturer's protocol. Gene-specific primer sequences are as follows: HPRT, 5′-tgaagagctactgtaatgatcagtc-3′ and 5′-agcaagcttgcaaccttaacca-3′; IL-17a, 5′-gctccagaaggccctcag-3′ and 5′-ctttccctcgcattgaca-3′; IL-23p19, 5′-agcgggacatatgaatctactaagaga-3′ and 5′-gtcctagtagggaggtgtgaagttg-3′; IL-17f, 5′-cccatgggattacaacatcactc-3′ and 5′-cactgggcctcagcgatc-3′; β-defensin2, 5′-aagtattggatacgaagcag-3′ and 5′-cactgggcctcagcgatc-3′ 5′-tggcagaaggaggacaaatg-3′.

2.6. Preparation of $CD4^+$ T cells and MLR

Spleen and lymph node (LN) cells of BALB/c mice were incubated with anti-CD4-coated MACS magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) and were positively selected. The purity of $CD4^+$ T cells was >95%, as determined by flow cytometry. $CD4^+$ T cells (2×10^5 cells) from BALB/c mice were co-cultured with each type of stimulated DCs (1×10^4 cells) in RPMI1640 medium. Cultures were performed at 37 °C (5% CO_2) for 60 h and pulsed with 3.7×10^4 Bq of [^{3}H]thymidine (Amersham Biosciences, GE Healthcare, Chalfont St. Giles, UK) during the final 12 h of incubation before harvesting. As a positive control, T cells were stimulated with 3 μg/ml of plate-bound anti-CD3 mAb (clone 145-2C11) and 0.5 μg/ml of soluble anti-CD28 mAb (eBioscience). [^{3}H]thymidine incorporation was determined on a microplate scintillation counter. For re-stimulation assay, expanded T cells (1×10^6 cells) were re-stimulated with plate-bound anti-CD3 Ab, and then cytokine production was assessed as described [15].

2.7. IL-23p19 luciferase reporter assay

The IL-23p19 promoter containing the genomic fragment −2302 to +12 of the IL-23p19 gene was amplified by PCR from C57BL/6 genomic DNA and cloned into the pGL4.26 vector (Promega, Madison, WI, USA). Luciferase assay was performed as described [13].

2.8. Isolation of lamina propria lymphocytes

Mice were orally administered with a suspension of Reishi (10 mg/body) or double distilled water using a sonde once every 2 days for 4 weeks. Mice were killed and intestines removed and placed in ice-cold PBS. After the removal of residual mesenteric fat tissue, Peyer's patches were carefully isolated, and the intestine was opened longitudinally. The intestine was then thoroughly washed and cut into 1.5-cm pieces. These pieces were incubated twice in 5 ml of 5 mM EDTA in HBSS for 15–20 min at 37 °C with slow rotation. After the second EDTA incubation, the pieces were washed in HBSS, cut into 1-mm^2 pieces using razor blades, and placed in 10 ml RPMI1640 medium digestion solution containing 4% fetal calf serum, 0.5 mg/ml Collagenase Type II (Invitrogen), 0.5 mg/ml DNase I (Sigma–Aldrich), and 1 mg/ml Dispase (Invitrogen). Digestion was performed by incubating the pieces at 37 °C for 20 min with slow rotation. After 20 min, the solution was vortexed intensely and passed through a 40-μm cell strainer. The pieces were collected and placed into fresh digestion solution, and the procedure was repeated a total of three times. Supernatants from all three digestions from each small intestine were combined, washed once in cold FACS buffer, resuspended in 10 ml of the 40% fraction of a 40:80 Percoll gradient, and overlaid on 5 ml of the 80% fraction. Percoll gradient separation was performed by centrifugation for 20 min at 2500 rpm at room temperature. Lamina propria lymphocytes (LPLs) were collected at the interphase of the Percoll gradient, washed once, and resuspended in FACS buffer. The cells were used immediately for experiments.

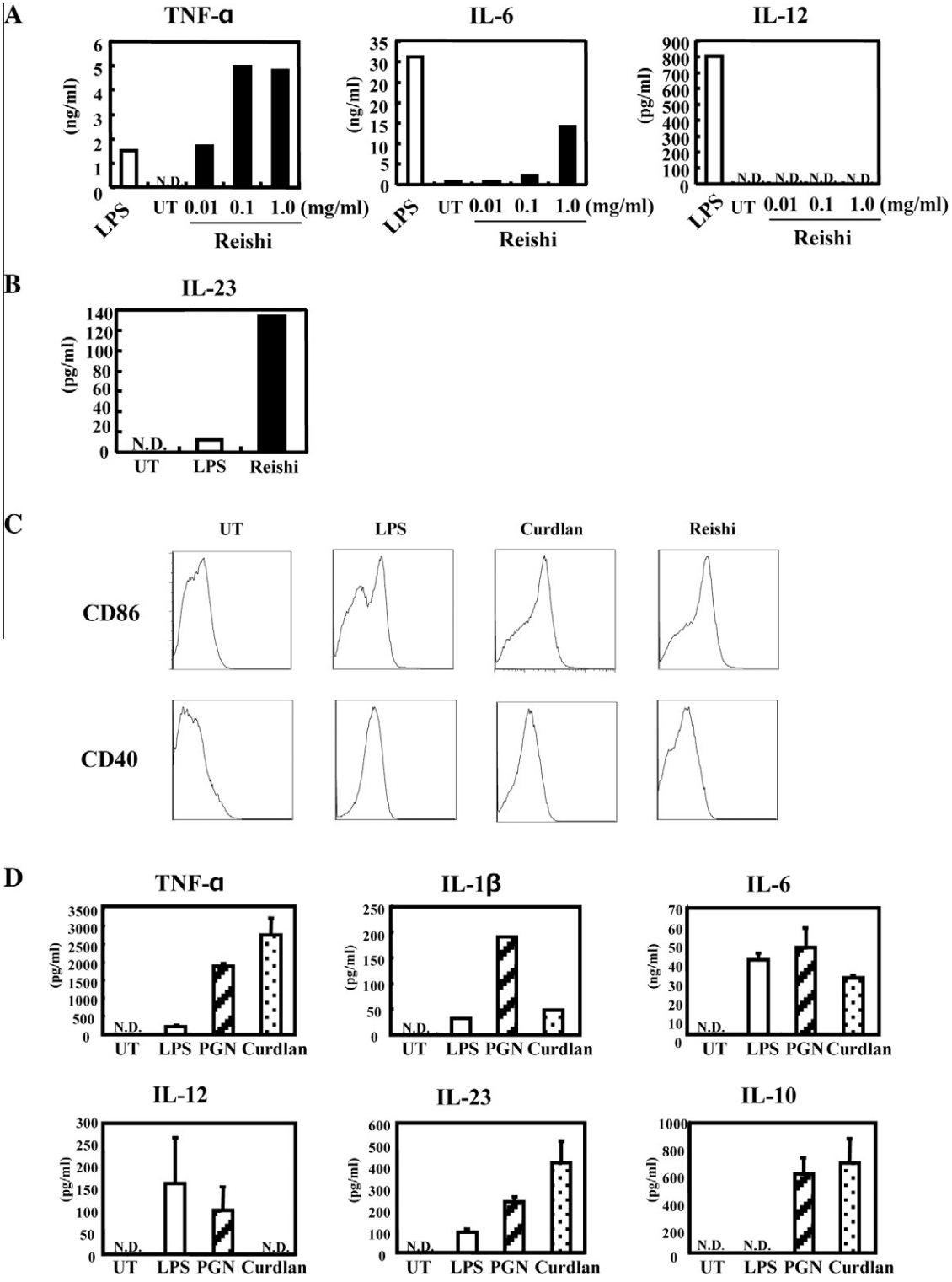

Fig. 1. Reishi induces pro-inflammatory cytokines in DCs. (A), (B) BMDCs were cultured for 12 h with LPS (100 ng/ml) or Reishi (0.01, 0.1, or 1 mg/ml) or without any stimulation (untreated; UT). The amounts of TNF-α, IL-6, IL-12 and IL-23 in the culture supernatants were measured by ELISA. N.D.; non-detectable. (C) BMDCs were cultured for 12 h with LPS (100 ng/ml), Curdlan (100 μg/ml), or Reishi (1 mg/ml), and flow cytometric analyses of the expression of CD40 and CD86 were performed. The results shown are representative of at least three independent experiments. (D) BMDCs were cultured for 12 h with LPS (100 ng/ml), PGN (100 μg/ml) or Curdlan (100 μg/ml). Cytokine levels in the culture supernatants were measured by ELISA.

3. Results

3.1. Reishi induces DCs to secrete abundant IL-23 but little or no IL-12

Reishi (*G. lucidum*) contains a variety of components [17]. We separated the components on the basis of their solubility in organic solvents such as EtOH and DMSO, and assessed each fraction's ability to produce inflammatory cytokines in mouse bone marrow-derived DCs (BMDCs). The fraction soluble in organic solvents did not induce any cytokine production in BMDCs (data not shown), while the fraction soluble in water [18], which contained the residue remaining after organic extraction, induced DC activation (Fig. 1).

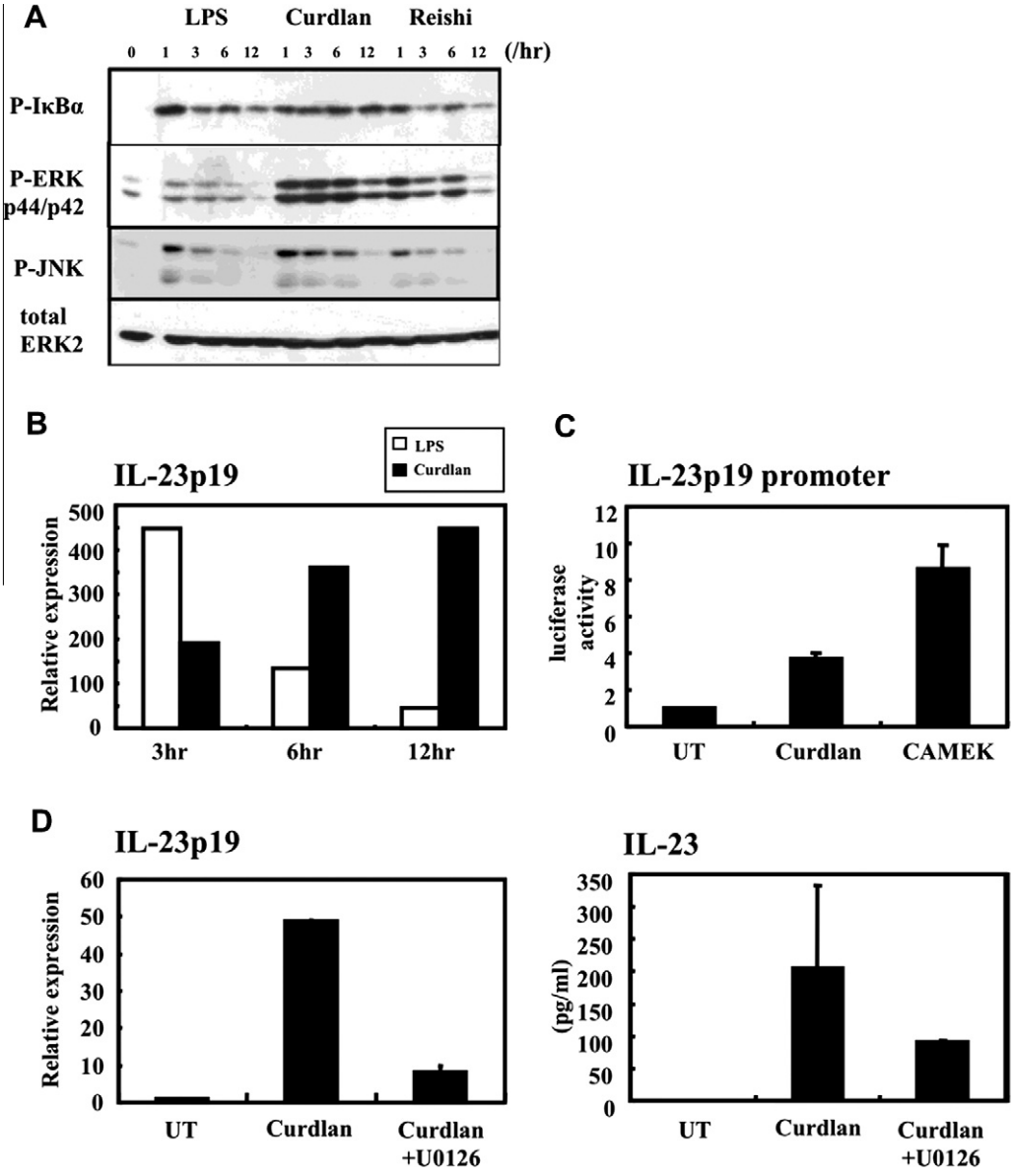

Fig. 2. Reishi induces IL-23 in DCs via a mechanism dependent on MEK-ERK. (A) BMDCs were stimulated with LPS (100 ng/ml), Curdlan (100 μg/ml) or Reishi (1 mg/ml) and then harvested at the indicated times, lysed and immunoblotted with the indicated antibodies. (B) BMDCs were stimulated with LPS (□) or Curdlan (■) and harvested at the indicated times. Total RNA was extracted, and quantitative RT-PCR for IL-23p19 mRNA was performed. All data were normalized to HPRT expression and are expressed as fold differences relative to those of unstimulated cells. (C) RAW264.7 cells were transfected with the IL-23p19 promoter plasmid with a control empty vector or an expression vector encoding constitutively active MEK (CAMEK). After 24 h, cells transfected with control vector were stimulated with Curdlan (1 μg/ml) for 6 h. Promoter activity was then measured and normalized to that of untreated cells (UT). (D) BMDCs were stimulated with Curdlan for 6 h in the presence or absence of U0126 (5 μM). Total RNA was harvested, and quantitative RT-PCR for IL-23p19 was performed. Data were normalized to HPRT expression and are expressed as fold differences relative to those of untreated cells (UT) (left). BMDCs were stimulated with Curdlan for 12 h in the presence or absence of U0126 (5 μM), and the amount of IL-23 in the culture supernatants was measured by ELISA (left). The results shown are representative of at least two independent experiments.

Thus we focused on the water soluble fraction thereafter, and referred to this fraction as Reishi.

In response to Reishi, DCs produced abundant TNF-α and IL-23, while they produced lower levels of IL-6, and barely detectable levels of IL-12 (Fig. 1A and B). In contrast, lipopolysaccharide (LPS)-stimulated DCs produced higher levels of IL-12 but not of IL-23 (Fig. 1A and B).

The pathogen-associated molecular patterns (PAMPs) that exist in the surfaces of the cell walls of fungi are reported to be recognized by DCs through TLR2, 4 and Dectin-1 [19,20]. Since the major components of Reishi are β-glucans and peptidoglycan (PGN), we compared the effects of β-glucan (Curdlan; β-1,3 linked glucans), PGN and LPS on DC activation (Fig. 1C and D). The costimulatory molecule CD86 as well as the maturation marker CD40 were upregulated in BMDCs cultured for 16 h with Curdan or Reishi, which response was similar to that achieved with LPS (Fig. 1C). Cytokine profiles of β-glucan-stimulated DCs were very similar to those of Reishi-stimulated DCs: they produced more IL-23 than IL-12. Curdlan also induced IL-10 from DCs. PGN showed an intermediate pattern between LPS and Curdlan. These data suggest that the main active ingredients in Reishi which is responsible for high IL-23 and low IL-12 production from DCs are the β-glucans.

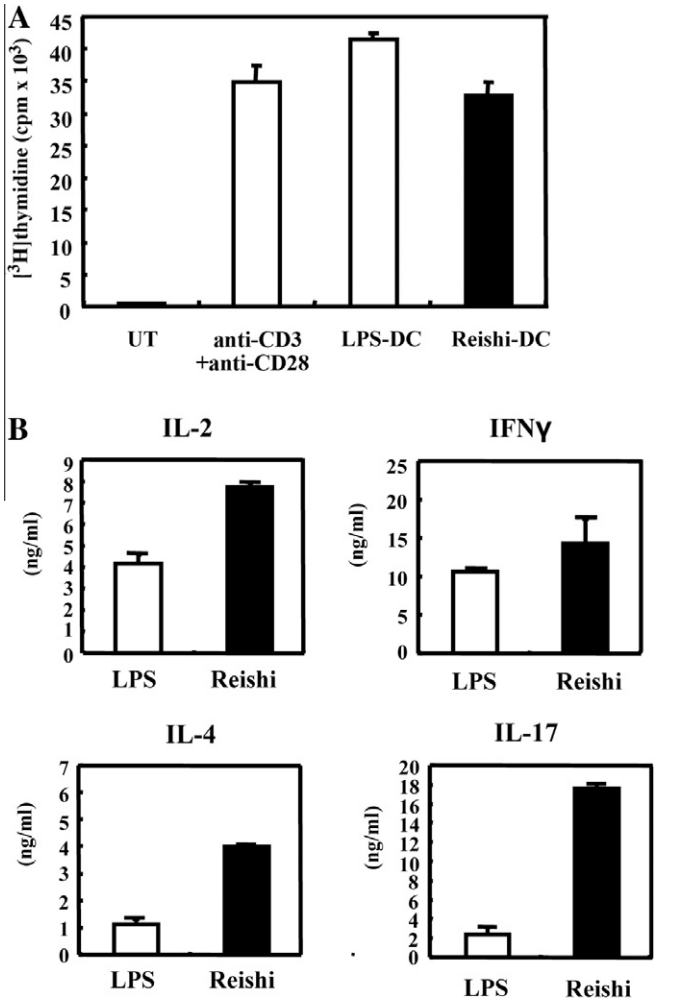

Fig. 3. Reishi-stimulated DCs induce Th17 cell development. (A) Allogenic $CD4^+$ T cells (2×10^5 cells) from BALB/c mice were co-cultured with LPS (100 ng/ml)- or Reishi (0.1 mg/ml)-stimulated BMDCs for 60 h (LPS-DC and Reishi-DC). Alternatively, T cells were stimulated with anti-CD3 and anti-CD28 mAbs (anti-CD3 + anti-CD28). Proliferation was measured by [^{3}H]thymidine incorporation. B) After 5 days, co-cultured T cells (1×10^6 cells) were restimulated with plate-coated anti-CD3 mAb for 12 h, and the amounts of IL-2, IFNγ, IL-4 and IL-17A in the culture supernatants were measured by ELISA. The results shown are representative of at least two independent experiments.

3.2. MEK-ERK signaling is involved in the induction of IL-23 in DCs by β-glucan stimulation

To clarify the molecular basis for the preferential induction of IL-23 by Reishi and β-glucan, we examined intracellular signaling pathways. As shown in Fig. 2A, Reishi and β-glucan induced a sustained phosphorylation of ERK1/2 (p44/p42) compared with LPS. Phosphorylation levels of JNK and IκBα induced by Reishi were similar to those by LPS (Fig. 1A).

IL-23 is a heterodimeric cytokine composed of a unique p19 subunit and a common p40 subunit shared with IL-12 [20]; IL-23p19 transcription levels were enhanced and sustained in β-glucan-stimulated DCs (Fig. 2B). Judged from the result of Fig. 2A, we speculated that ERK signaling is important for IL-23p19 transcription in response to β-glucan. To investigate this possibility, we cloned a IL-23p19 promoter and constructed a reporter system. After stimulation with β-glucan, IL-23p19 promoter activity was increased approximately 4-fold in RAW264.7 cells. Transfection of a constitutive active MEK, the upstream activator of ERK, into RAW264.7 resulted in a 10-fold upregulation of reporter activity (Fig. 2C). The MEK inhibitor U0126 profoundly reduced β-glucan-mediated IL-23p19mRNA induction (Fig. 2D, left). This was also confirmed by measuring the protein level using ELISA (Fig. 2D, right). These data indicate that the MEK-ERK pathway plays an essential role in the induction of IL-23 by β-glucan.

3.3. Reishi-treated DCs induce activation of T cells and differentiation of Th17 in vitro

Next, we evaluated the type of helper T cells generated in response to Reishi-stimulated DCs. We investigated the effects of Reishi-stimulated DCs on T cell proliferation and differentiation using an allogenic mixed lymphocyte reaction (MLR). LPS-stimulated DCs or Reishi-stimulated DCs from C57BL/6 mice were co-cultured with $CD4^+$ T cells from Balb/c mice. As shown in Fig. 3A, Reishi-stimulated DCs induced the proliferation of $CD4^+$ T cells, just as LPS-stimulated DCs did. To determine T cell differentiation, cytokine profiles in expanded T cells stimulated DCs were determined by ELISA. T cells cultured with Reishi-stimulated DCs produced large amounts of IL-17 but not of IFNγ than those cultured with LPS-stimulated DCs (Fig. 3B). Intracellular cytokine staining also confirmed an increase in Th17 cells in response to Reishi-stimulated DCs (data not shown). We also noticed that IL-4 production was enhanced in T cells after co-culture with Reishi-stimulated DCs. This suggests that Reishi-stimulated DCs have a potential to induce not only Th17 but also Th2 differentiation.

3.4. Reishi is a potent inducer of anti-microbial immunity via induction of Th17 cells in intestines

To determine whether Reishi could also induce a T cell response *in vivo*, we treated mice with Reishi by oral administration for 4 weeks and analyzed the population of Th17 cell populations in various organs by FACS. Reishi-treated mice had higher percentages of Th17 cells than water-treated mice in all types of gut-associated lymphoid tissue (GALT), excepting the MLN, but including the PP and LP (Fig. 4A). Interestingly, the population of $CD4^+$ $Foxp3^+$ (Treg) cells, which are specialized for immune suppression, also increased throughout the GALT, while the population of $CD4^-IFN\gamma^+$ cells increased only in the LP. Next, we examined IL-17-downstream molecules in the small intestines of Reishi-treated mice. IL-17A and IL-17F were recently indicated to be critical in inducing the expression of β-defensins, which are important for host defense [18]. The mRNA levels of both IL-17A and IL-17F, as well as β-defensins in the small intestines of Reishi-treated mice were higher than those in water-treated mice (Fig. 4B). These results suggest that Reishi might induce antibacterial activity via Th17 induction.

4. Discussion

In this study, we examined the effects of Reishi on activation of DCs and development of helper T cells. The results revealed that Reishi induces DCs to produce large amounts of IL-23 and thereby to develop Th17 cells *in vitro* and *in vivo*. Our data suggest that the main active ingredients in Reishi responsible for the activation of DCs are the β-glucans. As it has been reported elsewhere that β-glucans induce Th17 cell development [10], our results appear to be consistent with those reports. Reishi-treated DCs may also induce Th2 (Fig. 3B), however, precise mechanism remained to be clarified.

The metabolites of Reishi consist mainly of polysaccharides and terpenoids. After we separated the components of Reishi according to their solubility in organic solvents, the terpenoids should have been contained in the fraction that was soluble in organic solvents.

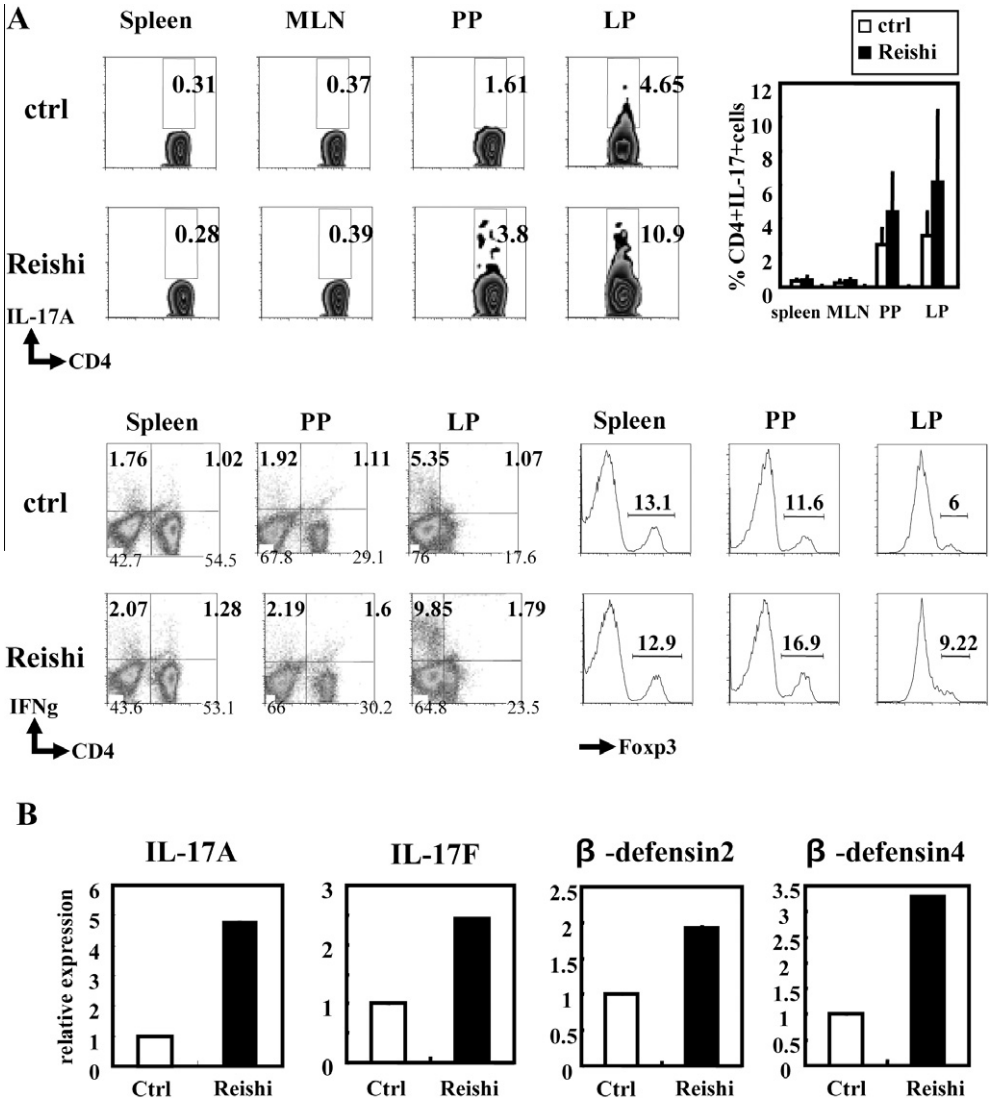

Fig. 4. Oral administration of Reishi increases Th17 cell population and antibacterial activity *in vivo*. (A) Mice were orally administered with a suspension of Reishi (10 mg/body) or double distilled water once every 2 days for 4 weeks, then intracellular flow cytometric analyses of the expression of IL-17A, IFNγ and Foxp3 were performed for splenocytes, mesenteric lymph node (MLN) cells, Peyer's Patch (PP) cells and Lamina Propria (LP) lymphocytes. The flow-cytometric plots are gated on $CD3^+$ cells (upper and middle-left). Numbers in the histograms are fractions of $CD4^+$ $Foxp3^+$ T cells (middle-right). Upper-right bar graph indicates the percentage of $CD4^+$ IL-$17A^+$ cells in three independent experiments. (B) After 4 weeks, total RNA of the small intestine was extracted, and quantitative RT-PCR was performed using the indicated primers. All data were normalized to HPRT expression and are expressed as fold differences relative to those from ddw-treated mice (ctrl).

In this study, the fraction that was soluble in organic solvents did not activate DCs; rather, it suppressed the production of TNF-α and IL-12 induced by LPS (data not shown). It has been reported elsewhere that triterpenoids and steroids from Reishi exhibited a potent inhibitory effect on NO production in LPS/IFNγ-stimulated microglial cells [21]. Additionally, the fruiting body of Reishi contains ergosterol, a precursor of vitamin D3 with known anti-inflammatory effects on DCs [22]. Thus the suppression of cytokine production by the fraction of Reishi that was soluble in organic solvents might also be due to terpenoids.

We showed that MEK-ERK signaling positively regulated IL-23p19 transcription in Reishi- and β-glucan-stimulated DCs. Kakimoto et al. reported that the Cot/Tpl2-ERK pathway is involved in the induction of IL-23p19 [23]. Recently, IL-23p19 expression during the response to TLR3 pathway activation was shown by promoter analysis to require the activation of ATF2 [24]. That study demonstrated that ATF2 binds directly to the IL-23p19 promoter region and activates its expression. The same mechanism may occur in Reishi-stimulated DCs. However, it is still unknown how the balance between IL-12 and IL-23 is regulated.

We found that the population of Th17 cells in GALT was increased in mice treated with orally-administered Reishi. Interestingly, the populations of $Foxp3^+$ Treg cells and $CD3^+$ $CD4^-$ IFNγ producing cells were also increased. As noted above, the fruiting body of Reishi contains ergosterol, which is a precursor of steroid hormones such as estrogen and vitamin D3. Previously, it has been shown that the priming of naïve $CD4^+CD25^-$ T cells with estrogen or vitamin D3 results in the generation of Treg cells [25,26]. Thus the increase in $Foxp3^+$ cells in the GALT of mice given Reishi orally might be caused by terpenoids rather than β-glucans. Additionally, Reishi-stimulated DCs produced IL-2, which may promote the proliferation of Treg cells. The resulting increase in Treg cells might prevent the excessive activation of immune cells that could otherwise be caused by Reishi. Alternatively, as it has recently been shown that Foxp3 + Tregs can be converted into Th17 cells in the presence of high levels of inflammatory cytokines, the high levels

of Treg cells may be related to the preferential Th17 development induced by Reishi [27]. We propose that the ability of Reishi to induce Th17 cells as well as Treg cells may contribute to the immunomodulatory effects of Reishi with regard to its effectiveness against tumors, allergies and atopic dermatitis.

Acknowledgments

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References

[1] T. Miyazaki, M. Nishijima, Studies on fungal polysaccharides. XXVII. Structural examination of a water-soluble, antitumor polysaccharide of *Ganoderma lucidum*, Chem. Pharm. Bull. 29 (1981) 3611–3616.
[2] Z.B. Lin, Cellular and molecular mechanisms of immuno-modulation by *Ganoderma lucidum*, J. Pharmacol. Sci. 99 (2005) 144–153.
[3] L.E. Harrington, R.D. Hatton, P.R. Mangan, H. Turner, T.L. Murphy, K.M. Murphy, C.T. Weaver, Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages, Nat. Immunol. 6 (2005) 1123–1132.
[4] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, H.L. Weiner, V.K. Kuchroo, Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells, Nature 441 (2006) 235–238.
[5] P. Ye, F.H. Rodriguez, S. Kanaly, K.L. Stocking, J. Schurr, P. Schwarzenberger, P. Oliver, W. Huang, P. Zhang, J. Zhang, J.E. Shellito, G.J. Bagby, S. Nelson, K. Charrier, J.J. Peschon, J.K. Kolls, Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense, J. Exp. Med 194 (2001) 519–527.
[6] S. Saijo, N. Fujikado, T. Furuta, S.H. Chung, H. Kotaki, K. Seki, K. Sudo, S. Akira, Y. Adachi, N. Ohno, T. Kinjo, K. Nakamura, K. Kawakami, Y. Iwakura, Dectin-1 is required for host defense against Pneumocystis carinii but not against *Candida albicans*, Nat. Immunol. 8 (2007) 39–46.
[7] S.M. Levitz, Th17 cells bounce off the fungal wall, Cell Host Microbe 5 (2009) 311–313.
[8] G.L. Stritesky, N. Yeh, M.H. Kaplan, IL-23 promotes maintenance but not commitment to the Th17 lineage, J. Immunol. 181 (2008) 5948–5955.
[9] M.J. McGeachy, Y. Chen, C.M. Tato, A. Laurence, B. Joyce-Shaikh, W.M. Blumenschein, T.K. McClanahan, J.J. O'Shea, D.J. Cua, The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo, Nat. Immunol. 10 (2009) 314–324.
[10] S. Saijo, Y. Iwakura, Dectin-1 and Dectin-2 in innate immunity against fungi, Int. Immunol. 23 (2011) 467–472.
[11] S. Saijo, S. Ikeda, K. Yamabe, S. Kakuta, H. Ishigame, A. Akitsu, N. Fujikado, T. Kusaka, S. Kubo, S.H. Chung, R. Komatsu, N. Miura, Y. Adachi, N. Ohno, K. Shibuya, N. Yamamoto, K. Kawakami, S. Yamasaki, T. Saito, S. Akira, Y. Iwakura, Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against *Candida albicans*, Immunity 32 (2010) 681–691.
[12] S. Vautier, G. Sousa Mda, G.D. Brown, C-type lectins, fungi and Th17 responses, Cytokine growth factor reviews 21 (2010) 405–412.
[13] K. Koga, G. Takaesu, R. Yoshida, M. Nakaya, T. Kobayashi, I. Kinjyo, A. Yoshimura, Cyclic adenosine monophosphate suppresses the transcription of proinflammatory cytokines via the phosphorylated c-Fos protein, Immunity 30 (2009) 372–383.
[14] T. Takimoto, Y. Wakabayashi, T. Sekiya, N. Inoue, R. Morita, K. Ichiyama, R. Takahashi, M. Asakawa, G. Muto, T. Mori, E. Hasegawa, S. Saika, T. Hara, M. Nomura, A. Yoshimura, Smad2 and Smad3 are redundantly essential for the TGF-beta-mediated regulation of regulatory T plasticity and Th1 development, J. Immunol. 185 (2010) 842–855.
[15] K. Ichiyama, T. Sekiya, N. Inoue, T. Tamiya, I. Kashiwagi, A. Kimura, R. Morita, G. Muto, T. Shichita, R. Takahashi, A. Yoshimura, Transcription factor Smad-independent T helper 17 cell induction by transforming-growth factor-beta is mediated by suppression of eomesodermin, Immunity 34 (2011) 741–754.
[16] T. Chinen, K. Komai, G. Muto, R. Morita, N. Inoue, H. Yoshida, T. Sekiya, R. Yoshida, K. Nakamura, R. Takayanagi, A. Yoshimura, Prostaglandin E2 and SOCS1 have a role in intestinal immune tolerance, Nat. Commun. 2 (2011) 190.
[17] Y.Y. Wang, K.H. Khoo, S.T. Chen, C.C. Lin, C.H. Wong, C.H. Lin, Studies on the immuno-modulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities, Bioorg. Med. Chem. 10 (2002) 1057–1062.
[18] R. Hanaoka, Y. Ueno, S. Tanaka, K. Nagai, T. Onitake, K. Yoshioka, K. Chayama, The water-soluble extract from cultured medium of *Ganoderma lucidum* (Reishi) mycelia (Designated as MAK) ameliorates murine colitis induced by trinitrobenzene sulphonic acid, Scand. J. Immunol. 74 (2011) 454–462.
[19] S. Akira, S. Uematsu, O. Takeuchi, Pathogen recognition and innate immunity, Cell 124 (2006) 783–801.
[20] G.D. Brown, S. Gordon, Immune recognition. A new receptor for beta-glucans, Nature 413 (2001) 36–37.
[21] H.H. Ko, C.F. Hung, J.P. Wang, C.N. Lin, Antiinflammatory triterpenoids and steroids from *Ganoderma lucidum* and *G. tsugae*, Phytochemistry 69 (2008) 234–239.
[22] A.E. Pedersen, M. Gad, M.R. Walter, M.H. Claesson, Induction of regulatory dendritic cells by dexamethasone and 1alpha,25-dihydroxyvitamin D(3), Immunol. Lett. 91 (2004) 63–69.
[23] K. Kakimoto, T. Musikacharoen, N. Chiba, K. Bandow, T. Ohnishi, T. Matsuguchi, Cot/Tpl2 regulates IL-23 p19 expression in LPS-stimulated macrophages through ERK activation, J. Physiol. Biochemist. 66 (2010) 47–53.
[24] F. Al-Salleeh, T.M. Petro, Promoter analysis reveals critical roles for SMAD-3 and ATF-2 in expression of IL-23 p19 in macrophages, J. Immunol. 181 (2008) 4523–4533.
[25] M.J. Polanczyk, B.D. Carson, S. Subramanian, M. Afentoulis, A.A. Vandenbark, S.F. Ziegler, H. Offner, Cutting edge: estrogen drives expansion of the $CD4^+CD25^+$ regulatory T cell compartment, J. Immunol. 173 (2004) 2227–2230.
[26] S. Joshi, L.C. Pantalena, X.K. Liu, S.L. Gaffen, H. Liu, C. Rohowsky-Kochan, K. Ichiyama, A. Yoshimura, L. Steinman, S. Christakos, S. Youssef, 1,25-Dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A, Mol. Cell. Biol. 31 (2011) 3653–3669.
[27] R. Takahashi, S. Nishimoto, G. Muto, T. Sekiya, T. Tamiya, A. Kimura, R. Morita, M. Asakawa, T. Chinen, A. Yoshimura, SOCS1 is essential for regulatory T cell functions by preventing loss of Foxp3 expression as well as IFN$^-${gamma} and IL-17A production, J. Exp. Med. 208 (2011) 2055–2067.