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Cerebroside D, a glycoceramide compound, improves experimental colitis in mice with multiple targets against activated T lymphocytes

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ABSTRACT

In the present paper, we aimed to examine the novel effects of cerebroside D, a glycoceramide compound, on murine experimental colitis. Cerebroside D significantly reduced the weight loss, mortality rate and alleviated the macroscopic and microscopic appearances of colitis induced by dexran sulfate sodium. This compound also decreased the levels of TNF-α, IFN-γ and IL-1β in intestinal tissue of mice with experimental colitis in a concentration-dependent manner, accompanied with markedly increased serum level of IL-10. Cerebroside D inhibited proliferation and induced apoptosis of T cells activated by concanavalin A or anti-CD3 plus anti-CD28 antibodies. The compound did not show an effect on naive lymphocytes but prevented cells from entering S phase and G2/M phase during T cells activation. Moreover, the treatment of cerebroside D led to apoptosis of activated T cells with the cleavage of caspase 3, 9, 12 and PARP. These results showed multiple effects of cerebroside D against activated T cells for a novel approach to treatment of colonic inflammation.

Introduction

Inflammatory bowel diseases are a group of chronic, multifactorial inflammatory disorders characterized by cytokine-driven and T lymphocyte-dependent inflammation of the intestinal mucosa (Thomas and Baumgart, 2012; Villablanca et al., 2011). They have a high incidence of 24.5/ 100,000 in western countries (Lakatos and Lakatos, 2006), not only impacting quality of life but also increasing the risk of colon cancer. Although the etiology of the disease is unknown, it has been suggested that the activation of the mucosal immune system in response to bacterial antigens with consecutive pathologic cytokine production plays a key pathogenic role (Salim and Soderholm, 2011). Moreover, the mucosa of patients with inflammatory bowel diseases is dominated by $CD4^+$ T lymphocytes, which is distinguished by its capacity of producing interferon-γ (IFN-γ) and interleukin-2 (IL-2) (Atreya et al., 2011; Sarra et al., 2010; Strober and Fuss, 2011). To mimic this disease in mice, a chemical-induced model of colonic inflammation has been introduced by orally delivering dexran sulfate sodium (DSS) and characterized by transmural inflammation associated with weight loss and histopathologic features that mimic some clinical demonstrations of inflammatory bowel diseases.

During screening of a variety of compounds, it was found that cerebroside D, a glycoceramide compound isolated from the culture of endophytic fungus — *Fusarium* sp. IFB-121, had potent immunomodulatory activities. Cerebrosides are a family of glycosphingolipids as important compounds in a wide variety of tissues and organs in biological systems (Tan and Chen, 2003). They were even found in Japanese Sake (Mitsuyama, 1983). Chemically, cerebrosides are composed of a hexose and a ceramide moiety usually consisting of a long-chain aminoalcohol trivially called 'sphingoid base' (=sphingosine or sphingol) and an amide-linked long-chain fatty acid. Although cerebroside D has been isolated previously from the methanol extract of fungi *Lactarium volemus* and *Pachybasium* sp. as early as 1988 (Sitrin et al., 1988), its biological activity is seldom reported, except for some antinociceptive activity (Koyama et al., 2002). In the present study, cerebroside D was found to improve inflammatory bowel diseases and this effect was mainly contributed by its targeting to activated T cells from multiple aspects including regulating cytokine profiles and inducing apoptosis.

Materials and methods

Drugs and reagents. The following drugs and reagents were used: cerebroside D (1-O-β-D-Glucopyranosyl-(2S,3R,4E,8E)-2-[(2R)-2-hydroxyoctadecanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol; more than 98% of purity), RPMI-1640 and FBS were purchased from Invitrogen (Carlsbad, CA). Anti-STAT1 (9H2) and anti-phospho-STAT1 were purchased from Cell Signaling Technology (Beverly, MA). Anti-cleaved caspase 3, caspase 9, caspase 12, PARP and anti-Actin were

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purchased from Santa Cruz Biotechnology (Santa Cruz, CA). ELISA kits for murine IL-2, TNF-α, IL-10 and IFN-γ were purchased from Dakewe Biotech Co. Ltd (Shenzhen, China). LDH assay kit was purchased from Promega (Madison, WI). Mouse T cell enrichment column was purchased from R&D systems (Minneapolis, MN). Cyclosporine A (CsA), dexran sulfate sodium (DSS), concanavalin A (Con A) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant murine IFN-γ was purchased from Peprotech (Rocky Hill, NJ). Annexin V-FITC (fluorescein isothiocyanate)/PI (propidium iodide) kit was purchased from BD Biosciences (San Jose, CA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Animals. Male C57BL/6 mice (6–8 weeks, 18–22 g) were obtained from the Shanghai Laboratory Animal Center (Shanghai, China) and group-housed at SPF facility under controlled temperature (22 ± 2 °C) and photoperiods (12:12-h light–dark cycle). Mice were allowed to acclimate to these conditions for at least 2 days before inclusion in experiments. For each group of experiments mice were matched by age and body weight. Care and experimentation of mice were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and the related ethical regulations of Nanjing University.

Induction and evaluation of colitis. Acute colitis was induced by feeding mice with 3% (wt/vol) DSS (molecular weight 40 kDa) dissolved in drinking water continuously from day 0 to day 5. Normal C57BL/6 mice received the same drinking water without DSS ($n = 10$ mice in each group). Cerebroside D (2.5, 5, 10 mg/kg) and CsA (10 mg/kg) were administered intraperitoneally once a day from day −3 to day 9. Animal were observed once daily for weight, water/food consumption, morbidity, stool consistency, and the presence of gross blood in feces and at the anus. The disease activity index (DAI) was calculated by assigning well-established and validated scores as previously described (Alex et al., 2009). Briefly, the following parameters were used for calculation: a) diarrhea (0 points = normal, 2 points = loose stools, 4 points = watery diarrhea); b) hematochezia (0 points = no bleeding, 2, slight bleeding, 4 points, gross bleeding). At day 9 following induction with DSS, animals were sacrificed, rapidly dissected, and the entire colon was quickly removed and macroscopic scores were blindly determined. Segments of the colon taken for histopathological essay were fixed in 10% normal buffered formalin, embedded in paraffin, sectioned at 4-μm thickness with a paraffin microtome, and mounted on microscope slides. Sections were stained with hematoxylin and eosin and histological score was calculated evaluating the damages of epithelium mucosa and inflammatory infiltration. Macroscopic and histological grading was done according to previously described (Appleyard and Wallace, 1995). A maximum score of 8 indicated severe colitis with overall diffuse pattern of chronic changes.

Analysis of cytokine profiles in colons and serum. At the time of mice sacrifice, serum was obtained. Colons from mice in each group were also homogenated with lysis buffer to extract total protein. Then the levels of cytokines IL-1β, TNF-α, IFN-γ and IL-10 were determined using the ELISA kits as described by the manufacturer.

Measurement of cell proliferation. Lymph node cells isolated from mice were incubated in 96 well-plate at a density of 5×10^5/ml cells per well in RPMI 1640 medium (0.2 ml) and stimulated with 5 μg/ml of Con A or 2 μg/ml anti-CD3/anti-CD28 for 72 h. The cells were cultured with or without various concentrations of cerebroside D. Then cell growth was evaluated with modified MTT assay. For MTT assay, 20 μl of MTT (4 mg/ml in PBS) was added per well 4 h before the end of incubation. MTT formazan production was dissolved by DMSO replacing the medium. The optical density at 570 nm (OD570) was measured by a microplate reader.

Cytotoxicity test. Lymph node cells (5×10^5) were incubated for 24 h or 48 h in the presence of various concentration of cerebroside D, respectively. LDH levels in the supernatants were measured as described by the manufacturer.

Measurement of cell cycle. For the essay of cell cycle, T cells purified from lymph nodes were treated with or without various concentrations of cerebroside D in the presence of Con A (5 μg/ml). Then cells were stained with propidium iodide (PI) and analyzed by flow cytometry after 24 h of stimulation.

Cell apoptosis assay. Cell apoptosis was determined by Annexin V/PI staining. The cells were measured by flow cytometry after addition of FITC-conjugated Annexin V and PI as previously described (Luo et al., 2011). Annexin V^+ cells were considered as apoptotic cells.

Western blot analysis. Western blot was done as described before (Sun et al., 2010). Briefly, the cells were collected and lysed in lysis buffer containing protease inhibitor (protease inhibitor cocktail, Pierce). The proteins were fractionated by SDS-PAGE and electrotransferred to PVDF membranes. Different antibodies were used for blotting, and detection was done by LumiGLO chemiluminescent system (KPL, Guildford, UK).

Statistical analysis. Results were expressed as mean ± s.e.m. of three independent experiments and each experiment included triplicate sets. Data were statistically evaluated by one-way ANOVA followed by Dunnett's test between control group and multiple dose groups. The level of significance was set at a P-value of 0.05.

Results

Cerebroside D attenuated DSS-induced colitis

Cerebroside D isolated from Fusarium sp. IFB-121 was subjected to HPLC analysis and structure determination. The purity of cerebroside D was confirmed to be over 98% by HPLC (Fig. 1). The structure of cerebroside D was identified by mass spectrometry and nuclear magnetic resonance (NMR) spectral analyses and compared with the reported data (Tan and Chen, 2003; Yue et al., 2001).

To examine the immunomodulatory activity of cerebroside D, we investigated the therapeutic efficacy of cerebroside D in DSS-induced T cell mediated murine colitis. DSS in mice induced a severe illness characterized by a dramatic loss of body weight and resulted in a high mortality rate. Compared with the control group, cerebroside D at 5 and 10 mg/kg recovered the body weight of mice to a normal level as CsA 10 mg/kg did. Increased survival rate of mice with this compound was dose-dependent (Figs. 2A and B). Disease progression in colitis model was also characterized by significant appearance of diarrhea/loose feces, and with visible fecal blood, resulting in significant DAI elevation. Comparatively, such changes were markedly improved by 5 and 10 mg/kg of cerebroside D and 10 mg/kg of CsA (Figs. 2C and D).

Cerebroside D inhibited pathological changes in DSS colitis mice

Morphological examination of DSS colitis revealed gross bleeding, ulceration and significant reduction in colon length. Watery feces were also observed. Compared to the control group, the mice treated with cerebroside D (10 mg/kg) only showed mild changes. CsA also alleviated the tissue damage (Figs. 3A and B).

Histological analysis showed that distortion of crypts, loss of goblet cells, infiltration of mononuclear cells, and severe mucosal damage in the colon specimens. However, these pathological changes were much improved after cerebroside D (10 mg/kg) treatment. The results of standard pathological tests in mice from cerebroside D-treated groups

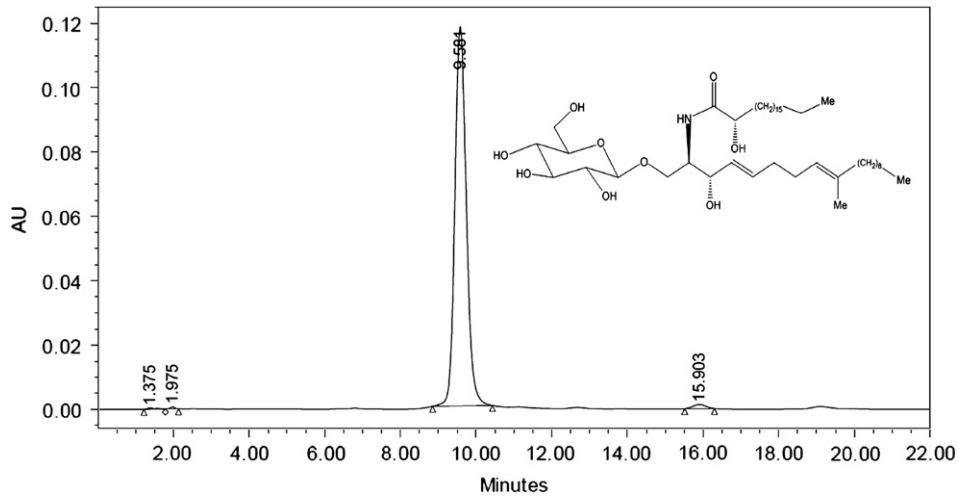

Fig. 1. HPLC analysis of cerebroside D and its chemical structure.

(5, 10, 20 mg/kg) have scores of 5.4, 2.9 and 2.1, respectively; while control mice exhibited a score of 7.8 (Figs. 3C and D).

Cerebroside D regulated the profiles of cytokines in colons of mice with DSS-induced colitis

To analyze the influence of the cytokines in acute DSS colitis model, levels of cytokines IFN-γ, TNF-α and IL-1β in colons and IL-10 in serum were measured in parallel following induction with colitis. Colon specimens from each group were picked out and homogenated for total protein extraction. Administration of cerebroside D or CsA to mice significantly suppressed inflammatory cytokines, including IFN-γ, TNF-α and IL-1β in colons (Figs. 4A–C). In addition, cerebroside D dose-dependently increased the level of the immunosuppressive factor IL-10 (Fig. 4D) in mice serum. All these changes in cytokines correlated with a significant suppression of inflammation and resolution of colitis.

Cerebroside D inhibited proliferation and caused G0/G1 arrest of T cells

Lymph nodes cells were incubated with or without cerebroside D for 72 h after the stimulation of Con A or anti-CD3/anti-CD28 for activating T cells. As shown in Fig. 5A, cerebroside D dose-dependently inhibited the proliferation of Con A-activated T cells, without affecting unactivated cells (Fig. 5C). A similar observation was made with lymph node cells activated by anti-CD3 plus anti-CD28 antibodies (Fig. 5B).

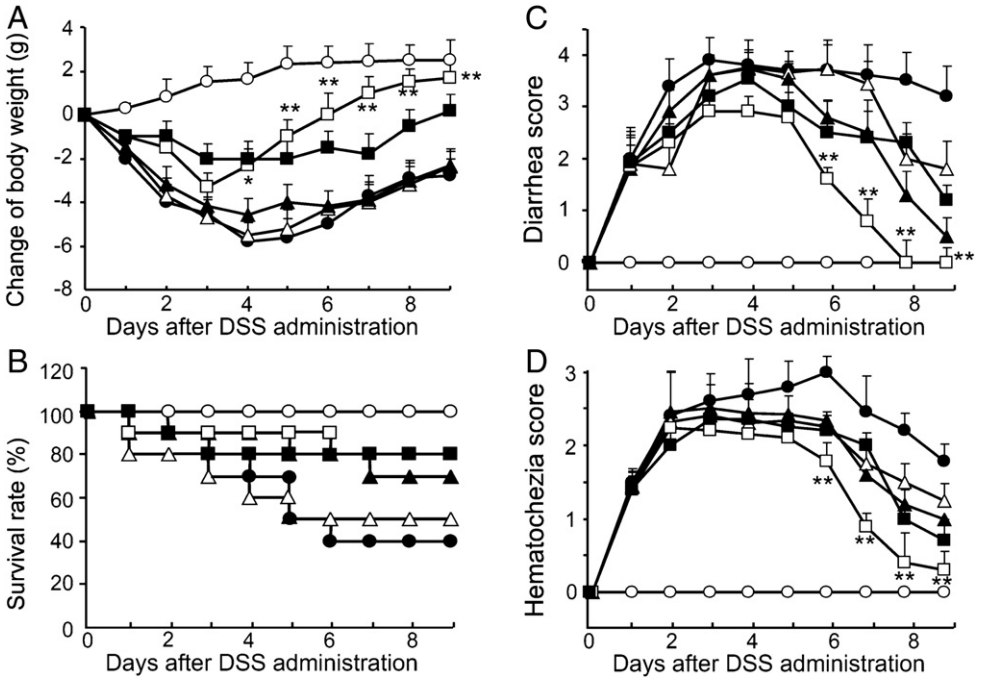

Fig. 2. Improvement of DSS-induced colitis by cerebroside D in mice. The colitis was induced by orally giving DSS from day 0 to day 5. Cerebroside D (2.5, 5 and 10 mg/kg) and cyclosporine A (10 mg/kg) were given intraperitoneally once a day from day 0 to day 9. Mice were sacrificed at day 9. (A) Body weight changes during the disease process. (B) Survival rate of mice. (C) & (D) Diarrhea and hematochezia evaluations of mice feces. ○, Control; ●, DSS treated; △, DSS + Cerebroside D 2.5 mg/kg;▲, DSS + Cerebroside D 5 mg/kg; □, DSS + Cerebroside D 10 mg/kg; ■, DSS + Cyclosporin A 10 mg/kg.

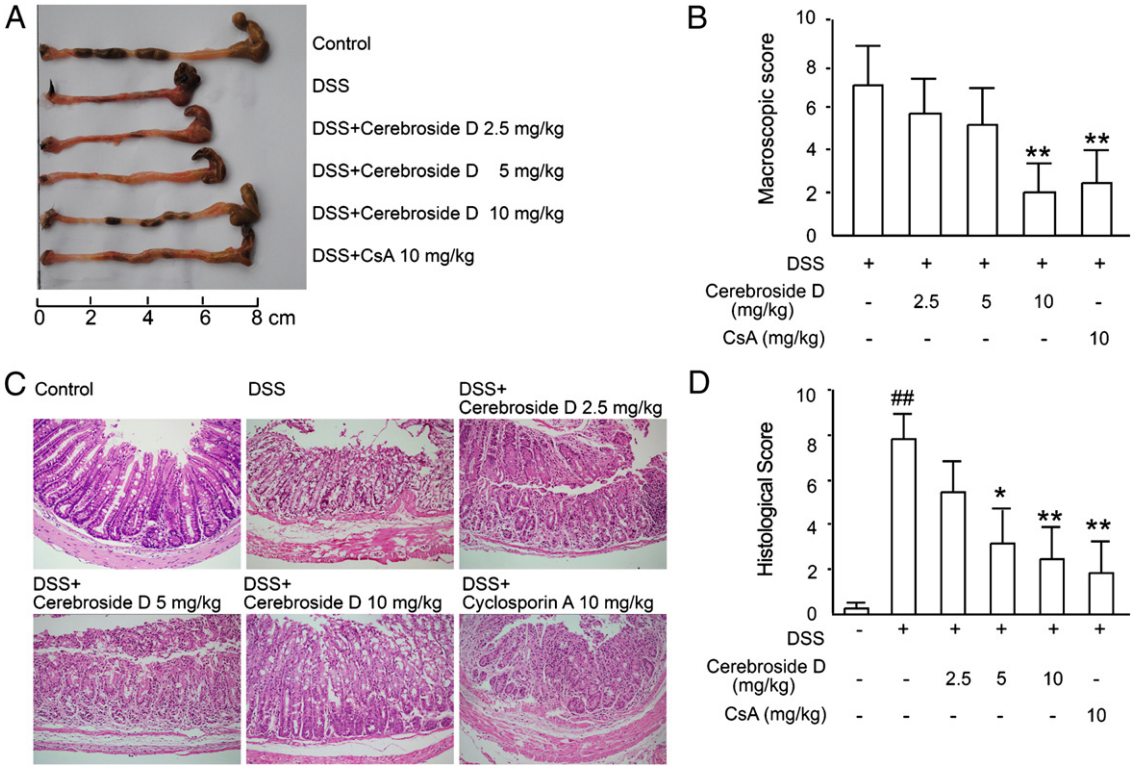

Fig. 3. Effects of cerebroside D and cyclosporine A on the macroscopic and microscopic appearances in mice colon with inflammation. Colons were taken out on the ninth day and the changes were examined as described in Materials and methods. (A) Macroscopic appearances of colons from DSS colitis mice. (B) Macroscopic evaluation of colons from DSS colitis mice. (C) Histopathological changes of colons. (D) Histopathological scores of colons from DSS colitis mice. The data indicated the mean ± s.e.m. of experimental animals ($n = 10$). One-way ANOVA revealed a significant difference at $P<0.01$. $^{*}P<0.05$, $^{**}P<0.01$, *vs.* DSS Control; $\#\#P<0.01$, *vs.* normal control (Dunnett's test). Histopathological sections were stained by H.E. The original amplification was 100× in (C).

In addition, T cells isolated from lymph nodes were initially resting in the G0/G1 phase of the cycle. Con A (5 μg/ml) stimulation resulted in the progression into the S phase and G2/M phase. Against this progression, cerebroside D showed an efficient inhibition (Fig. 5D).

Cerebroside D regulated of STAT1 signaling in T cells

Upon IFN-γ response of T cells, the phosphorylation but not the expression of STAT1 was remarkably inhibited by cerebroside D in a

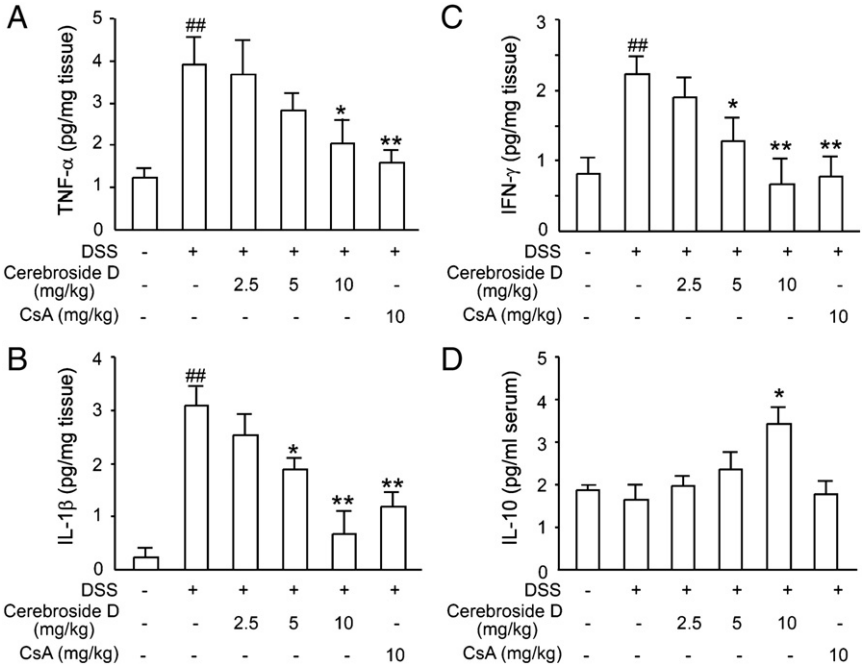

Fig. 4. Effects of cerebroside D on the production of inflammatory cytokines in colons or serum from mice with DSS-induced colitis. TNF-α (A), IL-1β (B), IFN-γ (C) in colons and IL-10 (D) in serum were determined by ELISA, respectively. One-way ANOVA revealed a significant difference at $P<0.01$. $^{*}P<0.05$, $^{**}P<0.01$, vs. DSS control; $\#\#P<0.01$, *vs.* normal control (Dunnet's test).

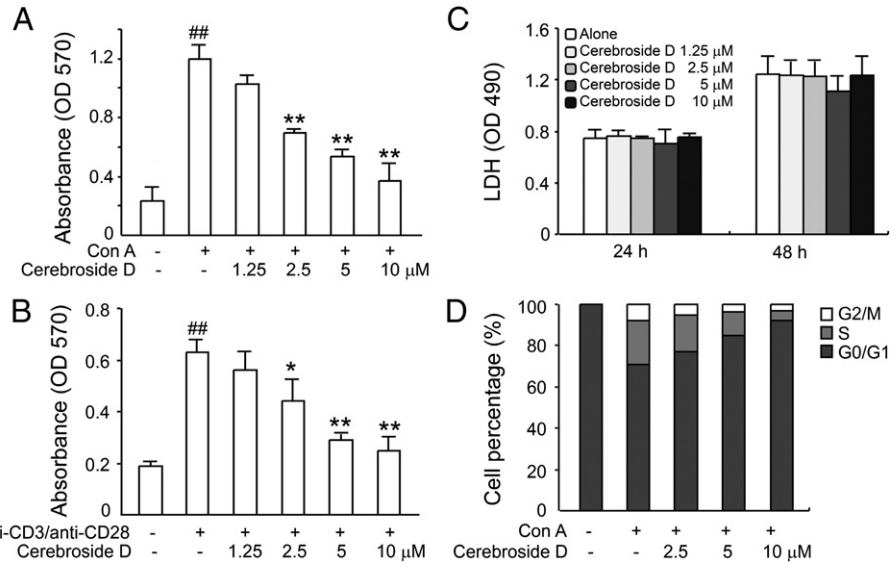

Fig. 5. Effect of cerebroside D on T lymphocyte proliferation induced by Con A or anti-CD3/anti-CD28. Lymph node cells (5×10^5) were incubated for 72 h at 37 °C and 5% CO_2 in the presence of 5 μg/ml Con A (A) or 2 μg/ml anti-CD3/anti-CD28 (B) as well as 1.25, 2.5, 5, 10 μM cerebroside D. Cell proliferation was measured at 570 nm by MTT uptake. (C) Cytotoxicity of cerebroside D on resting lymphocytes from normal mice. (D) Blockage of cell cycle in activated T cells by cerebroside D. T cells purified from lymph nodes were stimulated in the presence of Con A (5 μg/ml). Then cells were stained with propidium iodide (PI) and analyzed by flow cytometry after 24 h. One-way ANOVA revealed a significant difference at $P<0.01$. $^*P<0.05$, $^{**}P<0.01$, vs. Con A control; $\#\#P<0.01$, *vs.* normal control (Dunnet's test).

dose-dependent manner. In this response to IFN-γ, cerebroside D also reduced the expression of the IFN-γ-inducible gene T-bet in a dose-dependent fashion (Fig. 6).

Cerebroside D induced apoptosis of activated T lymphocytes

To examine the relationship of proliferation inhibition and apoptosis, we incubated T cells with cerebroside D in the presence of Con A for 48 h. Cell apoptosis was detected when concentrations higher than 1.25 μM were used (Fig. 7A). The T cells activated with Con A and exposed to cerebroside D underwent apoptosis in a dose-dependent manner (Figs. 7A and B).

To determine the potential mechanism of cerebroside D induced apoptosis, the expression of several apoptosis-related proteins was determined by Western blotting. After incubating with various concentrations of the compound (0, 1.25, 2.5, 5, 10 μM) at the presence of Con A (5 μg/ml) for 48 h, Caspase 3, 9, 12 and PARP in T cells were found to be cleaved in a dose-dependent manner (Figs. 7C and D). These results indicated that cerebroside D may triggers apoptosis through both endoplasmic reticulum stress-mediated and intrinsic mitochondrion-dependent apoptotic pathway.

Discussion

The chronic inflammatory bowel disease is recognized as an important cause of gastrointestinal disease in children and adults. It occurs worldwide but is more common in western countries (Hendrickson et al., 2002). Animal models of intestinal inflammation have provided useful insight into the pathogenesis of this disease (Hendrickson et al., 2002; Kuhl et al., 2007; Lorenz et al., 2005). In the present study, we demonstrated that a fungal metabolite, cerebroside D, has a marked immunosuppressive activity, which improves DSS-induced colitis in mice. As a result, intraperitoneal administration of cerebroside D, at doses of 2.5–10 mg/kg, significantly recovered the decrease in body weights and raised the survival rate against DSS-induced colitis damage (Figs. 2 and 3). The pathological observation also showed that the diarrhea was notably alleviated in the chemical-treated groups. Meanwhile, cerebroside D administration notably prevented the inflammatory damage of colonic tissues, including the colon edema, crypt distortion, goblet cell loss,

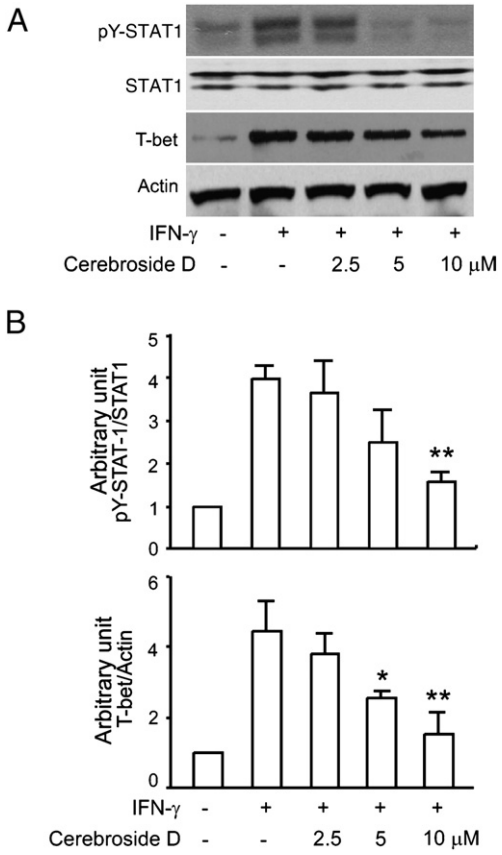

Fig. 6. Inhibition of STAT-1 phosphorylation and T-bet expression in lymphocytes by cerebroside D. T cells purified from lymph nodes (5×10^5) were incubated *in vitro*. (A) Western blot for pY-STAT1, STAT-1, T-bet and actin. The cells were treated with or without cerebroside D (2.5, 5, 10 μM) for 24 h followed by murine IFN-γ (25 ng/ml) incubation for 30 min. (B) Data summary are expressed as a histogram of mean ± s.e.m. of three independent experiments. Ratio of pY-STAT1/STAT1 and T-bet/Actin are shown. Data were statistically evaluated by one-way ANOVA followed by Dunnett's test between control group and multiple dose groups, with the level of significance chosen as $^*P<0.05$, $^{**}P<0.01$.

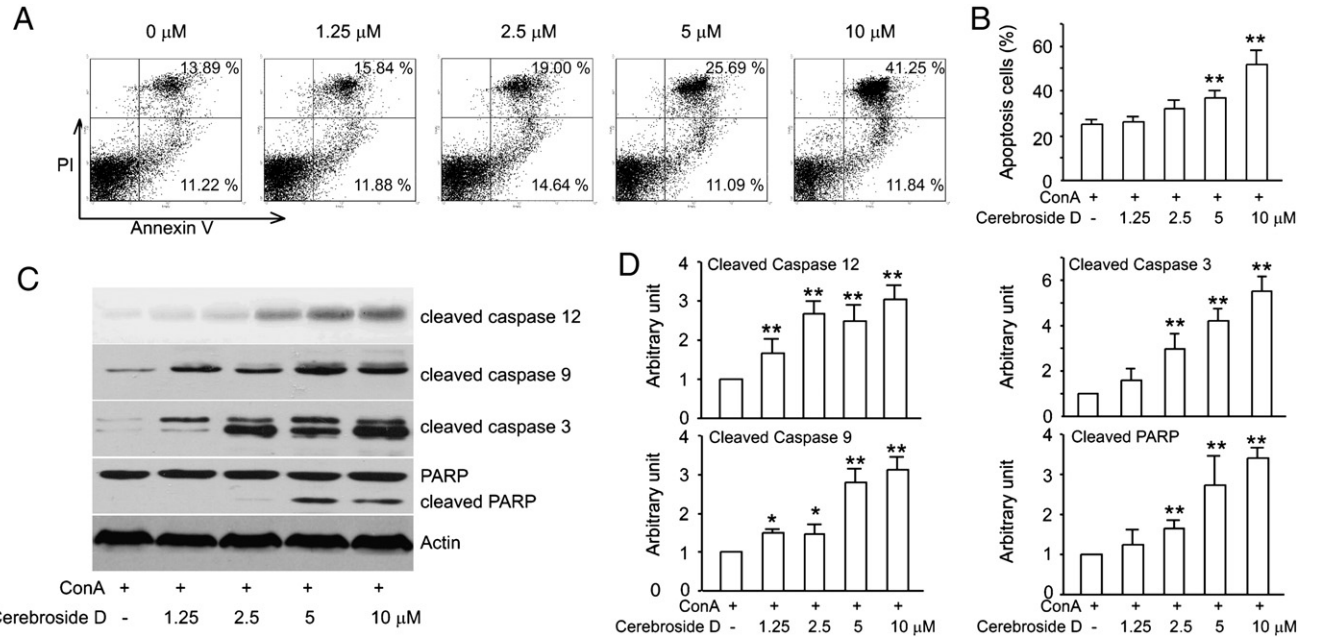

Fig. 7. Apoptosis of activated lymphocytes induced by cerebroside D. Cells were seeded in 6-well plate and incubated with 0, 1.25, 2.5, 5 and 10 μM cerebroside D for 24 h at the presence of ConA (5 μg/ml). (A) The apoptosis of T cells was determined by Annexin V/PI staining. (B) The values of apoptosis represented the mean ± SEM from three independent experiments. **P<0.01 vs. drug-untreated group. (C) Lymph node-derived T cells were treated by with 0, 1.25, 2.5, 5 and 10μM cerebroside D for 24 h. The protein levels of cleaved caspase 3, 9, 12 and PARP were determined by western blotting. The results shown are representative of three experiments. (D) The relative band intensity was analyzed and the values represented the mean ± SEM from three independent experiments. *P<0.05, **P<0.01 vs. drug-untreated group

mononuclear cell infiltration, etc. (Fig. 3). These findings suggest that it may be beneficial for the treatment of chronic inflammation diseases.

As we know, the T cell population that mediates the intestinal inflammation appears to be $CD4^+$ lymphocytes that secrete IL-2, IFN-γ and TNF-α (Guri et al., 2010; Kuhl et al., 2007; Peng et al., 2010). In depth characterization indicates that the stereotypical Th1 cytokines activate macrophages, which in turn, produce IL-1β, IL-12, IL-18 and other inflammatory factors and thus further stimulate T cells in a self-sustaining cycle (Loher et al., 2004; Neuman, 2004; Siegmund et al., 2001). We then assessed cytokine profiles in serum and colons of the DSS-induced colitis animals. The suppressive efficacy of cerebroside D on IL-1β, IFN-γ and TNF-α is important to elucidate its effects on colitis (Fig. 4). Furthermore, IFN-γ/STAT1/T-bet signaling is largely involved in the T cell-mediated immune response. In this study, inhibition of STAT1/T-bet signaling in T cells by cerebroside D may be linked to the resolution of DSS-induced colitis (Fig. 6). On the other hand, we found the level of serum IL-10 in colitis mice treated by cerebroside D increased significantly, which was unlike that in mice treated by CsA. IL-10 is a pluripotent cytokine considered as an immunoregulatory cytokine and its application is effective for the therapy of established and exacerbated immune diseases, such as psoriasis, inflammatory bowel diseases, and rheumatoid arthritis (Ishida, 1994; Kawamoto et al., 2008). So the increase in serum IL-10 in cerebroside D treated group further assured its anti-inflammatory efficacy. These findings suggest that cerebroside D has a unique property to modulate the cytokine profiles, reducing the pro-inflammatory cytokines and increase the anti-inflammatory cytokine in mice with colitis. Such characteristics of the compound may be very important for the treatment of inflammatory bowel diseases.

In addition, cerebroside D reduced the proliferative response of T cells activated by Con A or anti-CD3/anti-CD28 and had hardly any toxicity on nonactivated cells. Cerebroside D prevented T cells from entering S phase and G2/M phase after Con A stimulation (Fig. 5), suggesting that cerebroside D could interfere with naive T cell activation.

Meanwhile, cerebroside D induced activated T cell-apoptosis in a dose-dependent manner as demonstrated by Annexin/PI staining. It was well documented that intestinal T cells exhibit resistance to multiple apoptotic signals in experimental models of colitis, as well as in IBD patients (Monteleone et al., 2002; Neurath et al., 2001). This broad resistance to apoptosis accords with the fact that T cells in inflamed tissue express increased levels of Bcl-2 and thus may be resistant to a range of apoptotic mechanisms that involve mitochondrial activity (Hausmann et al., 2011; Liu et al., 2011; Tischner et al., 2012). Three different death signaling pathways lead to apoptosis, such as the extrinsic death receptor-dependent pathway, the intrinsic mitochondria-dependent pathway and the intrinsic ER stress-mediated pathway. These pathways work together to regulate the function of T lymphocytes (Dunkle and He, 2011; Shah et al., 2011). Cerebroside D demonstrated effects on the activation of caspases 3, 9 and 12 (Fig. 7). So it is likely that both ER stress and mitochondria mediated pathway was involved in cerebroside D-induced apoptosis. Since activation of T cells is a critical event in the pathogenesis of inflammatory bowel disease and facilitating apoptosis of activated T cells may provide a strategy for the treatment.

In summary, we explore the effects of a fungal metabolite, cerebroside D, on DSS-induced colitis. Its mechanism may involve multiple effects against activated T cells: regulation of the cytokine profiles, G0/G1 arrest of T cells, as well as an apoptosis induction in activated effector T cells. It should be noted that this compound did not affect the naïve lymphocytes, which may implicate a low toxicity profile. In the case presented here, targeting activated T cells from multiple aspects by cerebroside D, a glycoceramide compound from fungus culture, may contribute to a novel therapy for inflammatory intestinal diseases.

Conflict of interest statement

The authors declared no conflict of interest.

Acknowledgments

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References

Alex, P., Zachos, N.C., Nguyen, T., Gonzales, L., Chen, T.E., Conklin, L.S., Centola, M., Li, X., 2009. Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis. Inflamm. Bowel Dis. 15, 341–352.

Appleyard, C.B., Wallace, J.L., 1995. Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. Am. J. Physiol. 269, G119–G125.

Atreya, R., Zimmer, M., Bartsch, B., Waldner, M.J., Atreya, I., Neumann, H., Hildner, K., Hoffman, A., Kiesslich, R., Rink, A.D., et al., 2011. Antibodies against tumor necrosis factor (TNF) induce T-cell apoptosis in patients with inflammatory bowel diseases via TNF receptor 2 and intestinal CD14 macrophages. Gastroenterology 141, 2026–2038.

Dunkle, A., He, Y.W., 2011. Apoptosis and autophagy in the regulation of T lymphocyte function. Immunol. Res. 49, 70–86.

Guri, A.J., Mohapatra, S.K., Horne II, W.T., Hontecillas, R., Bassaganya-Riera, J., 2010. The role of T cell PPAR gamma in mice with experimental inflammatory bowel disease. BMC Gastroenterol. 10, 60.

Hausmann, M., Leucht, K., Ploner, C., Kiessling, S., Villunger, A., Becker, H., Hofmann, C., Falk, W., Krebs, M., Kellermeier, S., et al., 2011. BCL-2 modifying factor (BMF) is a central regulator of anoikis in human intestinal epithelial cells. J. Biol. Chem. 286, 26533–26540.

Hendrickson, B.A., Gokhale, R., Cho, J.H., 2002. Clinical aspects and pathophysiology of inflammatory bowel disease. Clin. Microbiol. Rev. 15, 79–94.

Ishida, H., 1994. Clinical implication of IL-10 in patients with immune and inflammatory diseases. Rinsho Byori 42, 843–852.

Kawamoto, M., Matsui, E., Kaneko, H., Fukao, T., Teramoto, T., Kasahara, K., Kondo, N., 2008. IL-10 plays an important role as an immune-modulator in the pathogenesis of atopic diseases. Mol. Med. Rep. 1, 837–842.

Koyama, K., Akiba, M., Imaizumi, T., Kinoshita, K., Takahashi, K., Suzuki, A., Yano, S., Horie, S., Watanabe, K., 2002. Antinociceptive constituents of *Auricularia polytricha*. Planta Med. 68, 284–285.

Kuhl, A.A., Pawlowski, N.N., Grollich, K., Loddenkemper, C., Zeitz, M., Hoffmann, J.C., 2007. Aggravation of intestinal inflammation by depletion/deficiency of gammadelta T cells in different types of IBD animal models. J. Leukoc. Biol. 81, 168–175.

Lakatos, L., Lakatos, P.L., 2006. Is the incidence and prevalence of inflammatory bowel diseases increasing in Eastern Europe? Postgrad. Med. J. 82, 332–337.

Liu, D., Huang, X., Cheng, S., Tong, W., Wan, P., Guan, Y., Zhao, H., 2011. Regulation of sishen wan on Bax/Bcl-2 mRNA, Fas/FasL in colonic tissue from rats with colitis. Zhongguo Zhong Yao Za Zhi 36, 3484–3488.

Loher, F., Bauer, C., Landauer, N., Schmall, K., Siegmund, B., Lehr, H.A., Dauer, M., Schoenharting, M., Endres, S., Eigler, A., 2004. The interleukin-1 beta-converting enzyme inhibitor pralnacasan reduces dextran sulfate sodium-induced murine colitis and T helper 1 T-cell activation. J. Pharmacol. Exp. Ther. 308, 583–590.

Lorenz, R.G., McCracken, V.J., Elson, C.O., 2005. Animal models of intestinal inflammation: ineffective communication between coalition members. Springer Semin. Immunopathol. 27, 233–247.

Luo, Q., Gu, Y., Zheng, W., Wu, X., Gong, F., Gu, L., Sun, Y., Xu, Q., 2011. Erlotinib inhibits T-cell-mediated immune response via down-regulation of the c-Raf/ERK cascade and Akt signaling pathway. Toxicol. Appl. Pharmacol. 251, 130–136.

Mitsuyama, T., 1983. Purification and characterization of cerebroside sulfatase activator. Hokkaido Igaku Zasshi 58, 502–512.

Monteleone, I., Vavassori, P., Biancone, L., Monteleone, G., Pallone, F., 2002. Immunoregulation in the gut: success and failures in human disease. Gut 50 (Suppl. 3), III60–III64.

Neuman, M.G., 2004. Signaling for inflammation and repair in inflammatory bowel disease. Rom. J. Gastroenterol. 13, 309–316.

Neurath, M.F., Finotto, S., Fuss, I., Boirivant, M., Galle, P.R., Strober, W., 2001. Regulation of T-cell apoptosis in inflammatory bowel disease: to die or not to die, that is the mucosal question. Trends Immunol. 22, 21–26.

Peng, X.D., Wu, X.H., Chen, L.J., Wang, Z.L., Hu, X.H., Song, L.F., He, C.M., Luo, Y.F., Chen, Z.Z., Jin, K., et al., 2010. Inhibition of phosphoinositide 3-kinase ameliorates dextran sodium sulfate-induced colitis in mice. J. Pharmacol. Exp. Ther. 332, 46–56.

Salim, S.Y., Soderholm, J.D., 2011. Importance of disrupted intestinal barrier in inflammatory bowel diseases. Inflamm. Bowel Dis. 17, 362–381.

Sarra, M., Monteleone, I., Stolfi, C., Fantini, M.C., Sileri, P., Sica, G., Tersigni, R., Macdonald, T.T., Pallone, F., Monteleone, G., 2010. Interferon-gamma-expressing cells are a major source of interleukin-21 in inflammatory bowel diseases. Inflamm. Bowel Dis. 16, 1332–1339.

Shah, D., Aggarwal, A., Bhatnagar, A., Kiran, R., Wanchu, A., 2011. Association between T lymphocyte sub-sets apoptosis and peripheral blood mononuclear cells oxidative stress in systemic lupus erythematosus. Free Radic. Res. 45, 559–567.

Siegmund, B., Fantuzzi, G., Rieder, F., Gamboni-Robertson, F., Lehr, H.A., Hartmann, G., Dinarello, C.A., Endres, S., Eigler, A., 2001. Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281, R1264–R1273.

Sitrin, R.D., Chan, G., Dingerdissen, J., DeBrosse, C., Mehta, R., Roberts, G., Rottschaefer, S., Staiger, D., Valenta, J., Snader, K.M., et al., 1988. Isolation and structure determination of Pachybasium cerebrosides which potentiate the antifungal activity of aculeacin. J. Antibiot. (Tokyo) 41, 469–480.

Strober, W., Fuss, I.J., 2011. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology 140, 1756–1767.

Sun, Y., Wu, X.X., Yin, Y., Gong, F.Y., Shen, Y., Cai, T.T., Zhou, X.B., Wu, X.F., Xu, Q., 2010. Novel immunomodulatory properties of cirsilineol through selective inhibition of IFN-gamma signaling in a murine model of inflammatory bowel disease. Biochem. Pharmacol. 79, 229–238.

Tan, R.X., Chen, J.H., 2003. The cerebrosides. Nat. Prod. Rep. 20, 509–534.

Thomas, S., Baumgart, D.C., 2012. Targeting leukocyte migration and adhesion in Crohn's disease and ulcerative colitis. Inflammopharmacology 20, 1–18.

Tischner, D., Gaggl, I., Peschel, I., Kaufmann, M., Tuzlak, S., Drach, M., Thuille, N., Villunger, A., Jan Wiegers, G., 2012. Defective cell death signalling along the Bcl-2 regulated apoptosis pathway compromises Treg cell development and limits their functionality in mice. J. Autoimmun. 38, 59–69.

Villablanca, E.J., Cassani, B., von Andrian, U.H., Mora, J.R., 2011. Blocking lymphocyte localization to the gastrointestinal mucosa as a therapeutic strategy for inflammatory bowel diseases. Gastroenterology 140, 1776–1784.

Yue, J.M., Fan, C.Q., Xu, J., Sun, H.D., 2001. Novel ceramides from the fungus *Lactarium volemus*. J. Nat. Prod. 64, 1246–1248.