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ORIGINAL ARTICLE

Immunological alterations in lupus-prone autoimmune (NZB/NZW) F1 mice by mycelia Chinese medicinal fungus *Cordyceps sinensis*-induced redistributions of peripheral mononuclear T lymphocytes

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Abstract Mycelia products from wild-form *Cordyceps sinensis* could be constantly produced in a large scale and would be a better source of this herbal medicine. Our purpose was to investigate the immunological effects of an orally administered hot-water extract cultured mycelium of *C. sinensis* in lupus-prone (NZB/NZW) F1 hybrids. Forty female mice were divided into four groups and were given 2.4 mg/g/day oral doses of *C. sinensis* starting at three (group A), six (group B), or eight (group C) months of age, whereas the remaining group (group D) served as a control. Survival, proteinuria, and titers of anti-double-stranded DNA autoantibodies were evaluated. Treatment with *C. sinensis* resulted in increased survival, decreased proteinuria, and reduced titers of anti-double-stranded DNA antibody in groups A and B. Moreover, the mice in groups A and B showed significantly reduced percentages of $CD4^+$ T cells ($^*P < 0.05$) and increased percentages of $CD8^+$ T cells in peripheral blood mononuclear cells (PBMC) after *C. sinensis* administration. At 6 months of age, the proliferation rate of BrdU-incorporated spleen cells was significantly decreased after 48 and 72 h of *C. sinensis* treatment ($^{**}P < 0.01$) in group A of mice. In conclusions, early medication with *C. sinensis* induced the redistribution of PBMC and attenuated the disease severity of lupus in (NZB/NZW) F1 mice.

Keywords Lupus · *Cordyceps sinensis* · (NZB/NZW) F1 mice · Immune modulation

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Introduction

Cordyceps sinensis (Berk) Sacc. is set much value on traditional Chinese medicine. Composed of a parasitic *Cordyceps* fungus and its host, the larva of, *Hepialus armoricanus Oberthur* [1, 2], *Cordyceps sinensis* (*C. sinensis*) is a common ingredient of nourishing tonics used to replenish health. Chemical analysis has revealed that *C. sinensis* contains protein, glycosides, nucleosides, polysaccharides, fats, vitamin B_{12}, cordycepicacid, and ergosterol. Major chemical constituents in *Cordyceps* include nucleosides such as, adenosine, adenine, hypoxanthicine nucleoside, uracil, thymine, uridine, guanidine, thymidine, 3′-deoxyadenosine (also called cordycepin); steroids such as ergosterol peroxide, cholesteryl palmitate, eogosterol; polysaccharides such as galactomannan; alkanols such as D-mannitol (also called cordycepic acid). It also contains a large quantity of crude proteins, essential amino acids, multiple trace elements and some vitamins [3]. Medical research has expanded the uses of *C. sinensis*, as a curative agent. For example, extracts of this organism possess broad functions in stimulating the immune system, in inhibiting tumor growth, and in treating inflammation

and oxidation [4–8]. Practioners of traditional Chinese medicine believe that *C. sinensis* may have *immunomodulatory* effect on the human immune system depending on the *strain*-specific constitutes of cultured and fermented *C. sinensis* mycelia.

Systemic lupus erythematosus is an autoimmune disease characterized by multiorgan involvement and various manifestations, including the production of autoantibodies. Among the murine models of lupus (NZB/NZW) F1 hybrid mice have a phenotype most similar to that of human lupus. Extracts of wild *C. sinensis* were reported to improve survival and inhibits anti-ds DNA antibody production in lupus mice [9]. Because cultured mycelium might be easily reproduced, the purpose of our study was to investigate the effects of orally administered hot-water extract cultured mycelium (CM) of *C. sinensis* on early, middle and late stage onset of 3-, 6-, or 8-month-old (NZB/NZW) F1 hybrid mice.

Materials and methods

Mice

Forty female 1-month-old (NZB/NZW) F1 mice were purchased (The Jackson Laboratory; Bar Harbor, Maine, USA). All mice were bred under the specific pathogen-free condition at the Animal Resource Services Facility at the National Defense Medical Center and mice were followed by the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985). The mice were divided into four groups (10 mice/group). Three groups were given oral doses of cultured mycelia of *C. sinensis* 2.4 mg/g/day starting at 3 (group A, early stage), 6 (group B, middle stage), or 8 (group C, late stage) months of age. The remaining group (group D) served as a control.

C. sinensis cultured mycelia (CM)

Cultured mycelia from *C. sinensis* were obtained (Chang Gung Biotechnology Company, Taipei, Taiwan). The reliable species of *C. sinensis* from *H. armoricanus Oberthur* were selected and the high-tech fermented/low-temperature culturing procedure was used to produce and isolate the biologically active gradients. Mannitol, ribonucleosides, protein, fats, vitamin B_{12}, and ergosterol were thus obtained to make cultured mycelia (CM). The percentage of similarity between 18S and 25S rDNA base pairs was 100% with wild-type (Fig. 1). The extracts from cultured mycelia were tested for endotoxin (lipopolysaccharide) and peptidoglycan before large-scale production. A dose curve from 1 to 16 mg/g/day was performed. Cultured mycelia from *C. sinensis* were made up in water and the extracts were fed by oral gavage five times (Monday–Friday) a week starting at 3 months of age until 12 months of age. The control animals were fed with water to control for the effect of feeding.

Outcome measures

The mice were monitored daily and examined at 1-month intervals to assess survival, proteinuria, peripheral lymphocytes, and titers of anti-double-stranded (ds) DNA antibody.

Proteinuria

Urine samples were obtained every 2 weeks to measure its protein content (Bayer Labstix, Bayside Medical Supplies Inc., Ontario, Canada). A grade of 0, 1, 2, 3, 4, or 5 was given to urine protein concentrations of 0–30, 30–100, 100–300, 300–2,000, and >2,000 mg/dl, respectively. Severe proteinuria was defined as > 100 mg/dl (>2+).

Peripheral lymphocytes

Blood was obtained from all mice at 12 months of age by means of the retro-orbital venous plexus for fluorescence-activated cell sorting. Single-cell suspensions of peripheral blood mononuclear cells (PBMC) and spleenocytes (which were depleted of erythrocytes by 3-amino-9-ethylcarbazole lysis buffer) were prepared in phosphate-buffered saline (PBS) containing 0.1% (w/v) bovine serum albumin and 0.1% (w/v) NaN_3. Aliquots were individually stained using anti-CD3-fuorescein isothiocyanate, anti-CD19-phycoerythrin, anti-CD4-phycoerythrin, and anti-CD8-phycoerythrin monoclonal antibodies. The samples were then analyzed (FACScan; Becton Dickinson Richmond, CA, USA) using forward and side scatter to gate out dead cells and debris. Data were collected after treatment with CM.

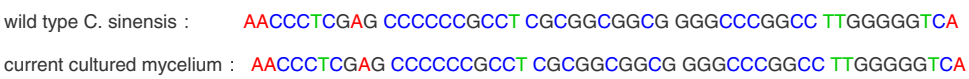


Fig. 1 Comparisons of first 50 (321–370) bases of the ITS2 rDNA sequence from wild-type *C. sinensis* and current cultured mycelia (CM) products

Cellular proliferation of DNA synthesis detection by BrdU enzyme-linked immunosorbent assay (ELISA)

The ELISA procedure was performed essentially as described by Roche Molecular Biochemicals. Briefly 5×10^3 cells/100 μl/well of splenocytes from mice groups A, B, C and D at 6-month-old were seeded in flat-bottom 96-well plates. A 10 μl/well of BrdU labeling solution was added to the cells after 24, 48 and 72 h. After three times of wash, anti-BrdU-POD-coupled antibodies were incubated in wells for 90 min at room temperature. The reaction was terminated by the addition of 0.05 ml of 5% (w/v) sodium dodecyl sulfate (SDS). Each sample was tested in triplicate. The results were read at 450 nm using a SPECTRAmax reader (Molecular Devices Corporation, Sunnyvale, CA, USA).

Autoantibodies

Blood was also obtained by means of the retro-orbital venous plexus to assess for anti-DNA antibodies. Sera for anti-DNA analysis were collected from whole blood in non-heparinized tubes. Immunoglobulin (Ig) G antibodies to dsDNA were assayed, as previously described [14]. In brief, calf thymus DNA (Sigma Chemicals, St Louis, MO, USA) was reconstituted to 0.5 mg/ml with PBS and frozen in aliquots at –20°C. DNA-coated plates were prepared, and microtiter plates were coated with 0.1 ml of 10 μg/ml methylated BSA (Sigma Chemical Co) in PBS and left overnight at 4°C. The plates were washed three times with PBS, 0.05 ml of 2.5 μg/ml dsDNA was added, and they were incubated overnight at 4°C. The plates were washed three times with PBS with 0.05% Tween-20 (PBS/Tween) and blocked with 0.1 ml of 1 mg/ml gelatin-PBS for 2 h at room temperature or overnight at 4°C. Serum was diluted 1:50 (previously determined to be the optimal dilution) in gelatin-PBS and added to each well in 0.05 ml aliquots. A standard curve was made by serial dilution of known concentrations of anti-DNA B cell hybridoma soup (generously donated by Dr. B.-L. Chiang, National Taiwan University, Taipei, Taiwan), and ELISA units, or E. U., were determined by comparing samples with one optimal dilution ratio of hybridoma supernatant. After the aliquots were incubated for 1–1.5 h at room temperature, they were washed three times with PBS/Tween. Horseradish peroxidase-conjugated goat anti-IgM or IgG (Jackson ImmunoResearch Laboratories, INC, Bar Harbor, Maine, USA) was added to gelatin-PBS at a 1:5,000 dilution and incubated for 1 h at room temperature. After three washes with PBS/Tween, the reaction was initiated with 50 ml of 0.55 mg/ml 2.2′-axino-bis-3-ethylbenthiazoline-6-sulfonic acid in citrate buffer (pH 4.2) containing 0.03% (v/v) H_2O_2. The reaction was terminated by adding 0.05 ml of 5% (w/v) sodium dodecyl sulfate. Each sample was tested in duplicate. Readings from gelatin-PBS represented background levels, and known positive and negative sera were used as controls. The results were read at 420 nm (SPECTRAmax reader; Molecular Devices Corporation, Sunnyvale, CA, USA).

Statistical analyses

Survival curves were estimated using the Kaplan–Meier method, and the curves were compared by means of Mantel–Cox analysis. Unpaired *t* test, one-way ANOVA were used to compare the differences between means of groups in the assay of proteinuria, flow cytometry, cellular proliferation and autoantibodies and statistical analysis was performed using SPSS.

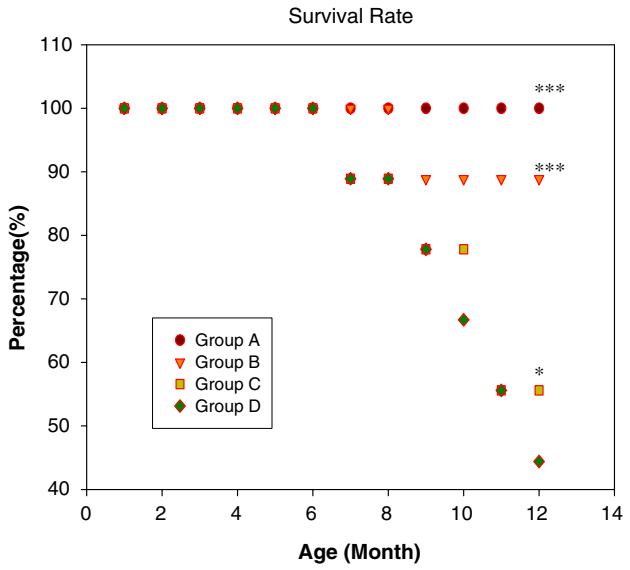
Results

Cultured mycelia (CM) from *C. sinensis* improved survival rates in groups A and B mice

Treatment with cultured mycelia (CM) from *C. sinensis* improved survival in early treatment of groups A and B mice. Figure 2 illustrates the survival curves (***$P < 0.001$ in groups A and B compared with group D at 12 months of age). All mice in groups A and B survived until 8 months of age, with a 90% survival rate at 12 months of age. In contrast, mortality was observed in groups C and D beginning at 7 months of age. These results indicated that the early prescription with cultured mycelia attenuated disease severity in autoimmune lupus-prone (NZB/NZW) F1 mice.

The severity of proteinuria was decreased in mice treated with cultured mycelia from *C. sinensis*

Early treatment with cultured mycelia (CM) from *C. sinensis* attenuated the severity of proteinuria in groups A and B mice (**$P < 0.01$ in groups A and B compared with group D at 12-month of age). Figure 3 illustrates the data regarding proteinuria. At 6 months of age, 60% of group D (control) mice had severe proteinuria; this rate increased to 80% at 10 months and 100% at 12 months. A similar pattern was seen in group C mice. However, proteinuria was more severe in group C mice at 8 and 9 months than in the control mice. In contrast, 50, 60, 60, and 60% of group A mice had severe proteinuria at 4, 6, 8, and 10 months of age, respectively. Before treatment with cultured mycelia, fewer mice in group B (40%) than in group A had severe proteinuria at 4 and 5 months of age.



Group A: CM treatment at 3-, 6-, 8- months of age
Group B: CM treatment at 6-, 8- months of age
Group C: CM treatment at 8- months of age
Group D: control mice

Fig. 2 Cultured mycelia (CM) from *C. sinensis* improved survival rates in groups A and B of mice. Comparison of survival curves in A, B, C and D groups of mice. Survival rate represents the percentage of mice survival on individual dates of months. The percentage of survival rate (***$P < 0.001$: groups A and B compared with group D at 12 months of age; *$P < 0.05$: group C compared with group D at 12 months of age)

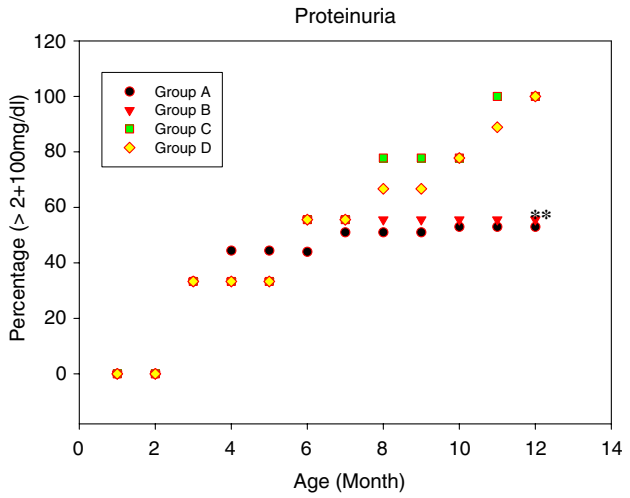


Fig. 3 The severity of proteinuria was decreased in mice treated with cultured mycelia (CM) from *C. sinensis*. Cumulative incidence of severe (>2+; 100 mg/dl) proteinuria in A, B, C and D groups of mice. Urine samples were obtained every 2 weeks for protein measurements by Labstix® (Bayer Labstix, Bayside Medical Supplies Inc., Ontario, Canada). (**$P < 0.01$: groups A and B compared with group D at 12 months of age)

Mice in groups A and B showed significantly reduced percentages of $CD4^+$ T cells in PBMC after *C. sinensis* administration

Mice in groups A and B had decreased percentages of $CD3^+$ T lymphocytes and increased percentages of $CD19^+$ B lymphocytes from peripheral mononuclear cells (PBMC) compared with percentages in the other groups (*$P < 0.05$) (Fig. 4). Regarding total T lymphocytes, groups A and B mice had fewer $CD4^+$ T cells (*$P < 0.05$) and mild higher levels of $CD8^+$ T cells than the other groups. However, group C mice, which received treatment with cultured mycelia at 8 months of age, had almost the same percentage of PMBC as that of the control mice at 12 months of age.

Serum levels of anti-dsDNA autoantibody were reduced after *C. sinensis* administration

Serum levels of anti-dsDNA autoantibody were reduced in the groups treated with cultured mycelia (CM) from *C. sinensis*. Figure 5 shows the serum levels of IgG anti-dsDNA antibody determined at 3–12 months of age in the treated and control groups. Treatment decreased levels of IgG anti-dsDNA autoantibody in group A at 6 months of age compared with groups B and C (**$P < 0.01$). Levels were also decreased in group B at 7 months of age immediately after the treatment. In contrast, group C, which received cultured mycelia at 8 months of age, had only a mild decrease in serum anti-dsDNA IgG levels immediately after treatment but continuous decrease till the end of 12 months of age. Most of group D (control) mice died because of lupus-like autoimmune disease, so that levels of IgG anti-dsDNA autoantibody were decreased at the end of 12 months of age.

C. sinensis reduced proliferation of splenocytes from group A mice at 6-month-old

Based on the observation that *C. sinensis* treatment decreased levels of IgG anti-dsDNA autoantibody in group A mice at 6 months of age compared with groups B and C (**$P < 0.01$), and with our unpublished data showed that *C. sinensis* treatment also mildly decreased the percentage of $CD4^+$ T cells from PBMC in group A mice compared with groups B and C at 6-month of age. Six months of age were chosen as the time point to measure the proliferation rate of peripheral T cells. Treatment with cultured mycelia (CM) from *C. sinensis* reduced proliferation rate in early treatment of group A mice. Cellular proliferation was detected after CM treatment by BrdU incorporation rate. The ratio compared with basal rate was significantly decreased in group A at either 48 or 72 h after BrdU

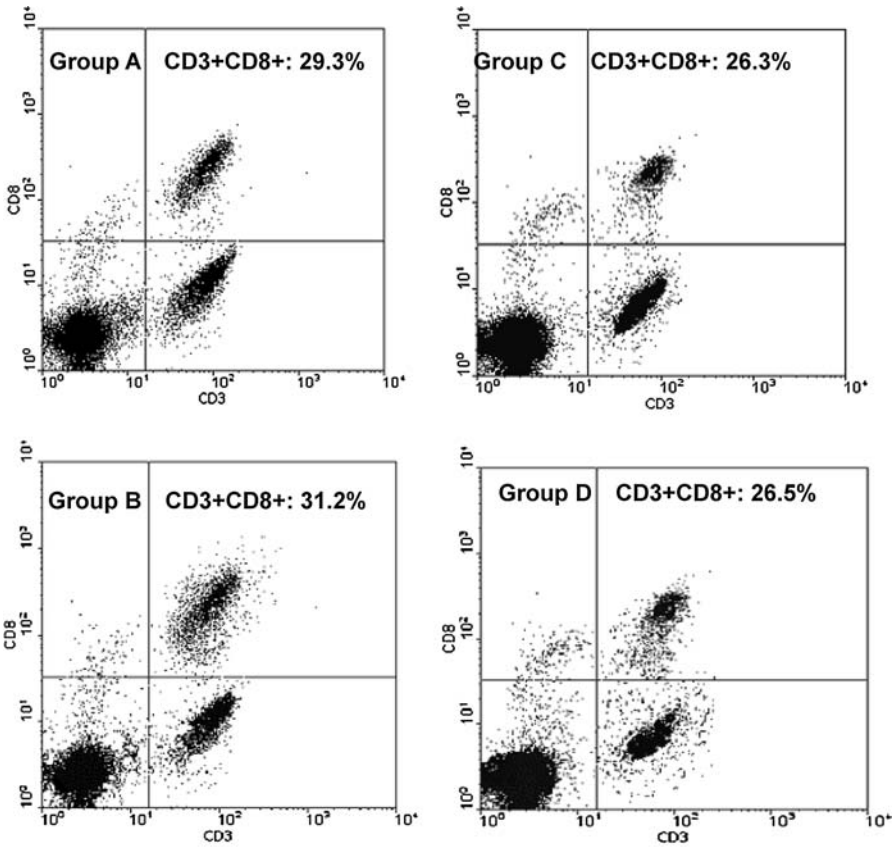


Fig. 4 Percentages of PBMC expressing $CD3^+$, $CD19^+$, $CD4^+/CD3^+$, and $CD8^+/CD3^+$ at 12 months of age.[a,b]

Group	$CD3^+$	$CD19^+$	$CD4^+/CD3^+$	$CD8^+/CD3^+$
A	50.45±3.15 [c]	41.15±3.55 [c]	21.16±2.46[c]	29.29±0.59
B	53.55±3.95 [c]	36.94±2.08 [c]	22.40±3.97[c]	31.15±1.76
C	65.07 ± 2.05	24.52 ± 1.59	38.73 ± 1.10	26.34 ± 0.57
D	63.80 ± 3.02	26.13 ± 0.96	37.25 ± 2.59	26.55 ± 0.72

[a] Analyzed by flow cytometry.

[b] Data is the means±standard deviation.

[c] *P < 0.05 for groups A and B compared with group D.

incorporation. $^{**}P < 0.01$ when comparing CM-exposed group A cells to groups B, C, and D cells was illustrated in Fig. 6.

Discussion

The effects of cultured mycelian therapy on autoimmune disease have been described. Many studies used wild extracts or fruiting bodies of *C. sinensis* as the sources for investigation. However, the bioactive ingredients existed might be different among daily extracts. Fermented mycelia can be constantly produced in a large scale and will be a better source of this herbal medicine. We examined the differential effects of temporal changes with cultured mycelium in lupus-prone mice during the early, middle and late stages of clinical disease. Treatment in the early and middle stages of disease development significantly increased the survival rate ($^{***}P < 0.001$).

The effect of *C. sinensis* treatment on murine lupus models, such as (NZB/NZW) F1 and MRL lpr/lpr mice has been studied. The data show changes in levels of antinuclear autoantibody levels in both strains; however, the temporal pattern of disease development was not detected.

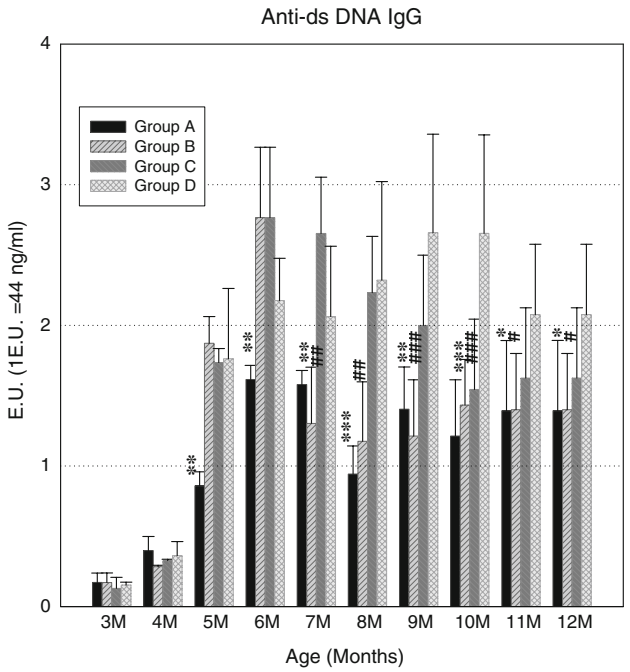


Fig. 5 Serum levels of anti-dsDNA autoantibody were reduced in *C. sinensis* treated groups. Comparisons of serum levels of IgG anti-dsDNA antibodies at 3–12 months of age were determined by enzyme-link immunosorbant assay (ELISA) after *C. sinensi* (CM) administration. Group *bar chart* with *error bars* showing serum levels of IgG anti-dsDNA antibodies at different months of age. Sera for anti-DNA analysis were collected with non-heparinized tubes using peripheral blood. Standard curve was made by serial dilution of anti-dsDNA autoantibody producing-B cell hybridoma supernatant. E.U. (ELISA unit) was determined by comparing samples with one optimal dilution ratio of hybridoma supernatant (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$: for group A compared with groups B, C and D at 5, 6 months of age; compared with groups C and D at 7–9 months of age and compared with group D at 10–12 months of age) ($^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#}P < 0.05$: for group B compared with groups C and D at 7, 8 months of age and compared with group D at 9–12 months of age)

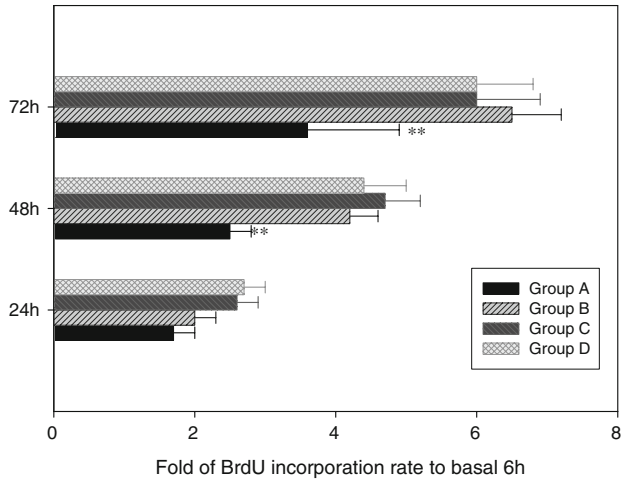


Fig. 6 Cultured mycelia (CM) from *C. sinensis* reduced proliferation of splenocytes from group A mice at 6-month-old. Cellular proliferation was detected after CM treatment by BrdU incorporation rate. A 5×10^3 cells/100 μl/well from each group of mice at 6-month-old were seeded in flat-bottom 96-well plates and cells were collected and incubated as basal (I). A 10-μl-well of BrdU labeling solution was added to the cells after 24 (II), 48 (III) and 72 h (IV). The time point values were compared against the basal level (time zero).The ratio was calculated using II, III and IV as dividend and I as divisor. **$P < 0.01$ when comparing CM-exposed group A cells to groups B, C, and D cells

For example, crude extracts of wild-type *C. sinensis* prolong the life span of female (NZB/NZW) F1 mice and inhibit the production of anti-dsDNA antibodies in vivo, but the level of proteinuria and the redistribution of lymphocytes on PBMC were not examined [9].

Yang et al. [10] isolated a pure compound (H1-A) from *C. sinensis* and investigated its effect on inhibiting the progression of autoimmune disease in MRL Ipr/Ipr mice. Mice treated daily with oral H1-A 40 μg/kg/day for 8 weeks had a progressive reduction in anti-dsDNA production when compared with the control group. In terms of clinical presentation, the treated group reduced lymphadenopathy, delayed progression of proteinuria, and improved kidney function. Histological analysis of kidney tissue indicated that H1-A could inhibit the mesangial proliferation evident in lupus-related nephritis. This may further support the argument that *C. sinensis* has immunomodulatory effects on autoimmune diseases. However, this animal model critically differs from ours with regard to disease pathogenesis and phenotype. MRL lpr/lpr mice have a defect of Fas receptor that causes lymphoproliferation and accelerated autoimmunity [11]. Therefore, the temporal pattern of disease development may differ between the models, yielding different end results after treatment with cultured mycelia. Our study of cultured mycelia from *C. sinensis* in (NZB/NZW) F1 mice significantly further support the potential use of *C. sinensis* on early prevention of lupus.

Autoreactive $CD4^+$ T cells have been recognized as a key factor in the pathogenesis of (NZB/NZW) F1 mice [12–15]. $CD4^+$ T cells can induce B cells to secrete cationic IgG anti-dsDNA antibodies, and they also stimulate autoreactive B cells to produce high-affinity pathogenic autoantibodies [16, 17]. Fluorescence-activated cell sorting analysis of PBMC demonstrated a reduced percentage of $CD4^+$ T lymphocytes in (NZB/NZW) F1 mice that received cultured mycelia in the early and middle phases. Moreover, the deposition of immune complex was significantly decreased during cultured mycelia therapy in our group A mice by immunofluorescent staining (our unpublished data). In mice, genetic deletion of B cells strongly suppresses systemic autoimmunity, providing a rationale for depleting B cells to treat autoimmunity. However, there

was an increase in B cells as well as the reduction in autoantibodies in our present study; perhaps, due to lower $CD4^+$ T lymphocytes and therefore less help to B cells [18, 19].

Regulatory T (Treg) cells induce immune tolerance by suppressing host immune responses against self- or non-self-antigens, thus, playing critical roles in the prevention of autoimmune diseases. As regulatory/suppressor T cells can suppress immunity against any antigen, they emerge as an ideal therapeutic target [20]. Several distinct subtypes of $CD8^+$ suppressor cells (Ts) have been described that could find application in treating RA or SLE [21]. In our study, groups A and B mice had fewer $CD4^+$ T cells ($^*P < 0.05$) and mild higher levels of $CD8^+$ T cells than groups C and D mice; it might be possible that cultured mycelia from *C. sinensis* could increase the activity of $CD8^+$ regulatory/suppressor T cells [22].

Although many studies have addressed cultured mycelium-induced immunomodulation, the mechanism remained unclear. H1-A inhibited tyrosine phosphorylation of human mesangial proteins, probably including Bcl-2 and Bcl-X_L [10]. These findings suggested that that H1-A modulate some subcellular signal-transduction pathways and changes the balance between proliferation and apoptosis of mesangial cells in vitro and in vivo. Our results suggested that the cultured mycelia-mediated immunosuppression target a particular cell population, decreasing the $CD4^+/CD8^+$ ratio, for example. The proliferation rate of BrdU-incorporated spleen cells was also significantly decreased after 48 and 72 h of CM treatment in group A mice. It has been demonstrated that the spleen cells from autoimmune-prone mice were more sensitive to oxygen and pressure compared with normal mice [23]. Future experiments will determine whether $CD4^+$ T cells lymphocytes in (NZB/NZW) F1 mice of lupus are particularly susceptible to apoptosis after treatment with cultured mycelia from *C. sinensis*.

Several classes of drugs are currently used to treat systemic lupus erythematosus; however, their clinically significant adverse effects pose considerable difficulties. *C. sinensis* since has been used as daily nourishing tonics is suggested for early prevention of lupus disease in our study. The current cultured mycelian products might potentially provide therapeutic and preventive benefits to patients with lupus.

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Conflict of interest statement The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

1. Zhu JS, Halpern GM, Jones K (1998) The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis* Part I. J Altern Complement Med 4(3):289–303
2. Zhu JS, Halpern GM, Jones K (1998) The scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis* Part II. J Altern Complement Med 4(4):429–457
3. Chen J, Chen T (2004) Chinese medical herbology and pharmacology. Art of Medicine Press, City of Industry, pp 883–885
4. Chen J, Zhang W, Lu T, Li J, Zheng Y, Kong L (2006) Morphological and genetic characterization of a cultivated *Cordyceps sinensis* fungus and its polysaccharide component possessing antioxidant property in H22 tumor-bearing mice. Life Sci 78(23):2742–2748
5. Jordan JL, Sullivan AM, Lee TD (2008) Immune activation by a sterile aqueous extract of *Cordyceps sinensis*: mechanism of action. Immunopharmacol Immunotoxicol 30(1):53–70
6. Lin CY, Ku FM, Kuo YC, Chen CF, Chen WP, Chen A, Shiao MS (1999) Inhibition of activated human mesangial cell proliferation by the natural product of *Cordyceps sinensis* (H1-A): an implication for treatment of IgA mesangial nephropathy. J Lab Clin Med 133(1):55–63
7. Kuo YC, Lin CY, Tsai WJ, Wu CL, Chen CF, Shiao MS (1994) Growth inhibitors against tumor cells in *Cordyceps sinensis* other than cordycepin and polysaccharides. Cancer Invest 12:611–615
8. Chen YJ, Shiao MS, Lee SS, Wang SY (1997) Effect of *Cordyceps sinensis* on the proliferation and differentiation of human leukemia U937 cells. Life Sci 60:2349–2359
9. Chen JR, Yen JH, Li CC, Tsai WJ, Liu WJ, Tsai JJ, Lin SF, Liu HW (1993) The effects of Chinese herbs on improving survival and inhibiting anti-ds DNA antibody production in lupus mice. Am J Chin Med 21(3–4):257–262
10. Yang LY, Chen A, Kuo YC, Lin CY (2003) H1-A extracted from *Cordyceps sinensis* suppresses the proliferation of human mesangial cells and promotes apoptosis, probably by inhibiting the tyrosine phosphorylation of Bcl-2 and Bcl-XL. J Lab Clin Med 141(1):74–83
11. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 356(6367):314–317
12. Wofsy D, Seaman WE (1985) Successful treatment of autoimmunity in NZB/NZW F1 mice with monoclonal antibody to L3T4. J Exp Med. 161(2):378–391
13. Sekigawa I, Ishida Y, Hirose S, Sato H, Shirai T (1986) Cellular basis of in vitro anti-DNA antibody production: evidence for T cell dependence of IgG-class anti-DNA antibody synthesis in the (NZB × NZW)F1 hybrid. J Immunol 136(4):1247–1252
14. Craft J, Peng S, Fujii T, Okada M, Fatenejad S (1999) Autoreactive T cells in murine lupus: origins and roles in autoantibody production. Immunol Res 19(2–3):245–257
15. Chen YC, Ye YL, Chiang BL (1997) Establishment and characterization of cloned CD4-CD8-alphabeta-T cell receptor (TCR)-bearing autoreactive T cells from autoimmune NZB × NZW F1 mice. Clin Exp Immunol 108(1):52–57
16. Ando DG, Sercarz EE, Hahn BH (1987) Mechanisms of T and B cell collaboration in the in vitro production of anti-DNA antibodies in the NZB/NZW F1 murine SLE model. J Immunol. 138(10):3185–3190
17. Datta SK, Patel H, Berry D (1987) Induction of a cationic shift in IgG anti-DNA autoantibodies: role of T helper cells with classical and novel phenotypes in three murine models of lupus nephritis. J Exp Med 165(5):1252–1268

18. Ahuja A, Shupe J, Dunn R, Kashgarian M, Kehry MR, Shlomchik MJ (2007) Depletion of B cells in murine lupus: efficacy and resistance. J Immunol 179(5):3351–3361
19. Fong KY (2002) Immunotherapy in autoimmune diseases. Ann Acad Med, Singapore 31(6):702–706
20. Suzuki M, Konya C, Goronzy JJ, Weyand CM (2008) Inhibitory CD8(+) T cells in autoimmune disease. Hum Immunol 69(11):781–789
21. Skaggs BJ, Singh RP, Hahn BH (2008) Induction of immune tolerance by activation of CD8(+) T suppressor/regulatory cells in lupus-prone mice. Hum Immunol 69(11):790–796
22. Jordan JL, Hirsch GM, Lee TD (2008) *C. sinensis* ablates allograft vasculopathy when used as an adjuvant therapy with cyclosporin A. Transpl Immunol 19(3–4):159–166
23. Chen YC, Chen SY, Ho PS, Lin CH, Cheng YY, Wang JK, Sytwu HY (2007) Apoptosis of T-leukemia and B-myeloma cancer cells induced by hyperbaric oxygen increased phosphorylation of p38 MAPK. Leuk Res 31(6):805–815