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FULL COMMUNICATION

Cordyceps militaris Enhances Cell-Mediated Immunity in Healthy Korean Men

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ABSTRACT *Cordyceps militaris* is a mushroom traditionally used for diverse pharmaceutical purposes in East Asia, including China, and has been found to be effective for enhancing immunity through various types of animal testing. The aim of this study is to determine the efficacy of *C. militaris* for enhancing cell-mediated immunity and its safety in healthy male adults. Healthy male adults were divided into the experimental group ($n = 39$), given 1.5 g/day of ethanol treated *C. militaris* in capsules, and the control group ($n = 40$), given the same number of identical placebo capsules filled with microcrystalline cellulose and lactose for 4 weeks from February 13 to March 14, 2012; the natural killer (NK) cell activity, lymphocyte proliferation index (PI), and T-helper cell 1 (Th1) cytokine cluster (interferon [IFN]-γ, interleukin [IL]-12, IL-2, and tumor necrosis factor [TNF]-α) were measured, along with stability test, at weeks 0, 2, and 4. The *C. militaris* group showed a statistically significant greater increase in NK200 ($P = .0010$), lymphocyte PI ($P \leq .0001$), IL-2 ($P = .0096$), and IFN-γ ($P = .0126$), compared with the basal level, than the placebo group. There was no statistically significant adverse reaction. *C. militaris* enhanced the NK cell activity and lymphocyte proliferation and partially increased Th1 cytokine secretion. Therefore, *C. militaris* is safe and effective for enhancing cell-mediated immunity of healthy male adults.

KEY WORDS: • *cordycepin* • Cordyceps militaris • *immune response*

INTRODUCTION

CORDYCEPS MILITARIS, an edible mushroom of the ascomycetes phylum, has been used for diverse purposes, including enhancement of immunity, activation of basal metabolism, recovery from fatigue, and improvement of liver and renal functions, mainly in East Asian regions, including Korea and China. Recently, its effectiveness for enhancing immunity has received increasing attention.[1–7]

Kim *et al.* observed that *C. militaris* enhanced spleen cell proliferation, natural killer (NK) cell activity, and secretion of T-helper cell 1 (Th1) cytokines, including interleukin (IL)-2, IL-12, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, in an immunosuppressed mouse. However, they reported that since it had no impact on T-helper cell 2 (Th2) cytokines, such as IL-4 and IL-10, *C. militaris* made stronger contributions to enhancement of cell-mediated immunity than to that of humoral immunity.[8]

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While diverse types of animal testing have been conducted on the contributions of *C. militaris* to immunity, almost no testing has been conducted in human subjects. The NK cell activity, cell proliferation, and the Th1 cytokine cluster were used as assessment indexes to determine the effectiveness of *C. militaris* to enhance immunity and to confirm its safety in healthy male adults.

MATERIALS AND METHODS

Specifications

C. militaris. *C. militaris* used in this study was supplied by Mushteck (Hoengseong, Korea).

Preparation of C. militaris. Dried *C. militaris* was crushed, extracted in 50% ethanol at room temperature and at normal pressure for 3 days, filtered, concentrated, sterilized, and spray dried; and then encapsulated with 375 mg *C. militaris* per capsule; placebo was manufactured with the same size and number of capsules filled with microcrystalline cellulose and lactose.

Bioactive components of C. militaris. The functional ingredient of *C. militaris* is cordycepin. The content of cordycepin is ~ 1.9 mg/g, acceptable within the range (80–120%).

Participants

This study was conducted to determine the immune-enhancing effects of *C. militaris* in healthy male adults who voluntarily agreed to participate in the study at Bundang Jesaeng General Hospital from February 13 to March 14, 2012. The participants were healthy men aged 19–64 who had abstained from drinking and smoking for 1 month and had taken no nutritional supplements, such as vitamins or lactic acid bacteria agents for 2 weeks. Those with a body–mass index lower than 18 or higher than 25, who had an uncontrolled systemic disease, such as rheumatoid arthritis, metabolic syndrome, autoimmune disease, or malignancy, infectious diseases, such as chronic hepatitis B or C or acquired immunodeficiency syndrome (AIDS), severe renal failure or hepatic failure, history of hypersensitivity to functional food, or were allergic to mushrooms, including *Cordyceps*, were excluded from the experiment. In addition, those thought to be unfit for the experiment, by the doctor, were also excluded.

Ethics consideration

The study was conducted under the approval of the institutional review board of Bundang Jesaeng General Hospital. The participants were given a full explanation of the purpose of the experiment, the efficacy of the experimental product, and its adverse reactions and gave written consent before starting the experiment.

Study design

The aim of this single-center, randomized, double-blinded placebo-controlled clinical trial was to determine the effects of *C. militaris* on lymphocyte-mediated cytotoxicity and immunity. The NK cell activity related to innate immunity, lymphocyte proliferation related to adaptive immunity, and the Th1 cytokine cluster (IFN-γ, IL-12, IL-2, and TNF-α) were measured to determine whether or not it was efficacious in enhancing immunity.

The adverse effects of the administration were examined to determine its safety and the clinical indexes, including systolic blood pressure, diastolic blood pressure, pulse rate, body temperature, and diagnostic blood tests (white blood cell, hemoglobin, hematocrit, platelet, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase, blood urea nitrogen, creatinine, and lactate dehydrogenase [LDH]), were used in the assessment.

An independent statistician unrelated to the clinical trial used the Proc Plan procedure of SAS (Ver. 9.1; SAS Institute, Cary, NC, USA) to randomly assign the participants before starting the trial.

The *C. militaris* group was given 1.5 g/day of *C. militaris* (two capsules per dose, twice per day) and the placebo group was given the same size and number of placebo capsules for 4 weeks from day 0 to the closing day. Blood sampling was performed thrice: before administration, after 2 weeks of administration, and after 4 weeks of administration. One subject dropped out of the *C. militaris* group, so that subject was eliminated from the statistical analysis.

Evaluation of immune enhancing effects

Cell separation. Peripheral blood mononuclear cells (PBMCs) from participants were isolated by density gradient centrifugation with Ficoll-Paque PLUS solution (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and used for the determination of cytotoxicity.

Cell line. The K-562, an erythroleukemic cell line developed by Lozzio and Lozzio,[9] was maintained in RPMI1640 (Flow Laboratories Ltd., Rickmansworth, United Kingdom) supplemented with 10% fetal bovine serum (Gibco, Paisley, United Kingdom), 3.0 mmol L-glutamine (Bio-Whittaker, Walkerswille, MD, USA), 100 mg/mL penicillin, and 100 mg/mL streptomycin at 37°C. Exponentially growing cells in 35-mm petri dishes were used in our study and viability was determined by trypan blue dye exclusion test.

Treatment of K-562 tumor cell line. K-562 cells were preincubated in petri dishes in a culture medium for 24 h. Cells were then washed and 100 μL of K-562 cells at a concentration 5.0×10^5/mL of culture medium were transferred into a new 96-microtiter plate. The LDH release was determined after 2, 4, and 6 h of culture of K-562 cells at 37°C in a humid atmosphere containing 5% CO_2.

LDH release cytotoxic assay. A modified LDH release assay was used to determine the PBMC cytotoxicity in the K-562 cell line.[10] PBMC as effector cells (100 μL) at a concentration of 1.0×10^7/mL, 5.0×10^6/mL, and 2.5×10^6/mL of culture medium were mixed with 100 μL of K-562 cells at a concentration of 5.0×10^5/mL, resulting in three effector-to-target (E:T) ratios of 200:1, 100:1, and 50:1, respectively. Each E:T ratio was determined in triplicate. The assay was performed in 96-microwell plates (Flow Laboratories Ltd.), which were incubated for 4 h at 37°C in a humid atmosphere with 5% CO_2. The plates were then centrifuged for 5 min at 200 *g*. Supernatants from each well (100 μL) were transferred into flat-bottom 96-microwell plates and 100 μL of lactic acid dehydrogenase substrate mixture was added. A microtiter plate reader (Behringer EL-311) was used for the evaluation of changes in absorbance at 492 ± 630 nm. The percentage of cytotoxicity was calculated with correction for the LDH release from PBMC using the formula as below:

$$\frac{\mathrm{LDH}_{\mathrm{experimental}}-\mathrm{LDH}_{\mathrm{effector\ cells}}-\mathrm{LDH}_{\mathrm{spontaneous}}}{\mathrm{LDH}_{\mathrm{maximal}}-\mathrm{LDH}_{\mathrm{spontaneous}}}\times100$$

where $\mathrm{LDH}_{\mathrm{experimental}}$ represents the LDH release activity resulting from cocultures of effector and target cells, $\mathrm{LDH}_{\mathrm{effector\ cells}}$ represents the released LDH activity from separately cultured effector cells, $\mathrm{LDH}_{\mathrm{spontaneous}}$ represents the activity released from cultures of K-562 cells, and

$LDH_{maximal}$ represents the LDH activity released from K-562 cells after lysis by sonication, thrice per 15 sec at 35 kHz.

NK50, NK100, and NK200 were used to express the released LDH cytotoxicity in E:T ratios of 50:1, 100:1, and 200:1, respectively.

Percentage of LDH release from K-562 cells. The percentage of permeability of LDH release through the cell membrane of K-562 cells was calculated from the spontaneous LDH release (expressed as absorbance, *A*) and the maximal LDH release (expressed as absorbance, *A*) using the following formula:

$$\%\text{LDH} = \frac{\text{LDH}_{\text{spontaneous}}(A)}{\text{LDH}_{\text{maximal}}(A)}$$

Cytokine assay. Once informed consent was obtained, blood samples were collected from the *C. militaris* and placebo groups on day 0, 14, and 28. Serum was separated and the cytokine assay for IL-2, IL-12, TNF-α, and IFN-γ was performed using the enzyme-linked immunosorbent assay with commercial assay kits (R&D Systems, Inc., Abingdon, United Kingdom). The absorbance of each well was read at 492 nm. Cytokine concentrations in the samples were calculated using a standard curve generated from recombinant cytokines. Cytokine values were expressed as pg/mL.[11]

Cell proliferation by MTT assay. PBMCs were purified from heparinized venous blood. Blood samples were collected just before the experiment. Cells were suspended in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. PBMCs (5×10^6 cells/mL) were seeded into 24-well plates for 2 days at 37°C in an atmosphere of 5% CO_2 in air; 100 μL aliquots of each cell suspension was added to the wells of sterile flat-bottom 96-well culture plates. Concanavalin A (Sigma–Aldrich, St. Louis, MO, USA) was added to the culture medium at the concentration of 10 μg/mL. Cell proliferation was assessed using a colorimetric MTT (3-[4, 5-Dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) proliferation kit (ATCC, Manassas, VA, USA). Four hours before the end of the assay, 10 μL of 5 mg/mL MTT was added to each well. The reaction was ended by the addition of 100 μL of dimethylsulfoxide to induce the dissolution of formazan crystals. The optical density (OD) of the developed color was read on a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 570 nm. The results of the lymphocyte proliferation assay are presented as the proliferation index (PI) [$\text{PI} = 100 \times (\text{OD}_{\text{stimulated culture}} - \text{OD}_{\text{negative control culture}})/\text{OD}_{\text{negative control culture}}$].[12–14]

Statistical analysis

Kruskal–Wallis ANOVA, Mann–Whitney test, and the Student's *t*-test were used for analysis of data. $P \leq .05$ was considered statistically significant.

RESULTS

Clinical characteristics of participants

Clinical characteristics and ages of the participants were not statistically significant between groups (age 36.58 ± 12.08 years for the *C. militaris* group and 36.50 ± 11.05 years for the placebo group). There was also no statistically significant intergroup difference in the mean weight and height of subjects (Table 1).

NK cell activity

The NK cell activity was measured before administration and after 4 weeks of administration to examine the variation in both groups.

NK50. The mean NK50 (E:T ratio 50:1) increased by an average of 3.97 ± 9.65, from 14.06 ± 5.80 before administration to 18.03 ± 6.85 after 4 weeks of administration in the *C. militaris* group and increased by an average of 0.38 ± 14.45, from 16.36 ± 10.27 to 16.75 ± 10.73 in the placebo group. NK50 showed a statistically significant increase in the *C. militaris* group ($P = .0071$) and a statistically

Table 1. General and Biochemical Characteristics of Study Participants

	Placebo group (n = *40*)	C. militaris *group* (n = *40*)	P *value*
Age (years)	36.50 ± 11.05	36.58 ± 12.08	.8463
Height (cm)	173.51 ± 5.82	174.15 ± 7.30	.6649
Weight (kg)	69.74 ± 9.02	69.75 ± 7.97	.9969
Vital signs			
Body temperature (°C)	36.15 ± 0.50	36.30 ± 0.37	.0689
SBP (mmHg)	124.15 ± 5.78	124.55 ± 6.68	.3877
DBP (mmHg)	77.98 ± 6.81	76.90 ± 6.39	.2345
Pulse rate (beat/min)	73.85 ± 8.09	74.03 ± 7.53	.4603
Laboratory findings			
White Blood Cell ($\times 10^3/mm^3$)	5.77 ± 1.52	6.00 ± 1.24	.2309
Hemoglobin (g/dL)	15.17 ± 0.99	14.92 ± 0.96	.1302
Hematocrit (%)	42.32 ± 8.33	45.30 ± 19.20	.1855
Platelet ($\times 10^3/mm^3$)	223.56 ± 55.42	221.20 ± 36.33	.4114
Aspartate aminotransferase (IU/L)	23.92 ± 6.74	23.80 ± 9.40	.4745
Alanine aminotransferase (IU/L)	23.94 ± 12.10	23.80 ± 13.90	.4809
Alkaline phosphatase (IU/L)	216.73 ± 61.45	212.53 ± 57.00	.3761
γ-Glutamyl transferase (IU/L)	32.35 ± 27.32	29.85 ± 22.58	.3284
Blood urea nitrogen (mg/dL)	13.83 ± 3.59	13.88 ± 3.41	.4734
Creatinine (mg/dL)	1.16 ± 0.11	1.12 ± 0.14	.1177
Lactate dehydrogenase (IU/L)	368.65 ± 55.45	436.63 ± 478.55	.1888

All data are expressed as mean ± SD or number (percentage) of participants. There was no significant difference in baseline characteristics between two groups.

P values by *t*-test.

C. militaris, *Cordyceps militaris*; SBP, systolic blood pressure; DBP, diastolic blood pressure.

insignificant increase in the placebo group ($P = .4337$) after the 4-week administration and there was no statistically significant intergroup difference in the NK50 variation ($P = .990$, Fig. 1).

NK100. The mean NK100 (E:T ratio 100:1) increased by an average of 4.30 ± 13.82, from 19.45 ± 9.75 before administration to 23.75 ± 10.72 after 4 weeks of administration in the *C. militaris* group ($P = .0299$) and increased by an average of 1.87 ± 10.85, from 17.09 ± 8.42 to 18.96 ± 6.82 in the placebo group ($P = .1406$). There was no statistically significant intergroup difference in the NK100 variation ($P = .1941$, Fig. 1).

NK200. The mean NK200 (E:T ratio 200:1) increased by an average of 10.69 ± 20.04, from 23.09 ± 10.35 before administration to 33.78 ± 16.23 after 4 weeks of administration in the *C. militaris* group ($P = .0010$) and increased by an average of 0.76 ± 12.78, from 23.99 ± 10.40 to 24.75 ± 8.71 in the placebo group ($P = .3547$). Significant intergroup differences in the NK200 variation were found after 4 weeks of administration ($P = .0055$, Fig. 1).

Th1 cytokine cluster

The Th1-originated mediator cluster (TNF-α, IL-12, IFN-γ, and IL-2) and cell proliferation were examined for measurements of the adaptive immune functions in the participants and their variation after 4 weeks of *C. militaris* (or placebo) administration was estimated.

IFN-γ. IFN-γ increased by 616.03 ± 1650.85 pg/mL, from 816.33 ± 636.0 pg/mL to 1432.37 ± 1304.86 pg/mL in the *C. militaris* group ($P = .0126$), but decreased by 12.82 ± 1318.67 pg/mL, from 1064.14 ± 823.10 pg/mL to 1051.32 ± 1062.16 pg/mL in the placebo group. Significant intergroup differences in IFN-γ variation were also found after 4 weeks of administration ($P = .0324$, Fig. 2).

IL-12. IL-12 increased by 0.97 ± 4.42 pg/mL, from 28.08 ± 3.58 pg/mL before administration to 29.05 ± 3.53 pg/mL after 4 weeks of administration in the *C. militaris* group and increased by 1.61 ± 7.08 pg/mL, from 28.44 ± 4.63 pg/mL to 30.05 ± 4.77 pg/mL in the placebo group. There was no significant intergroup difference in the IL-12 variation ($P = .3160$, Fig. 2).

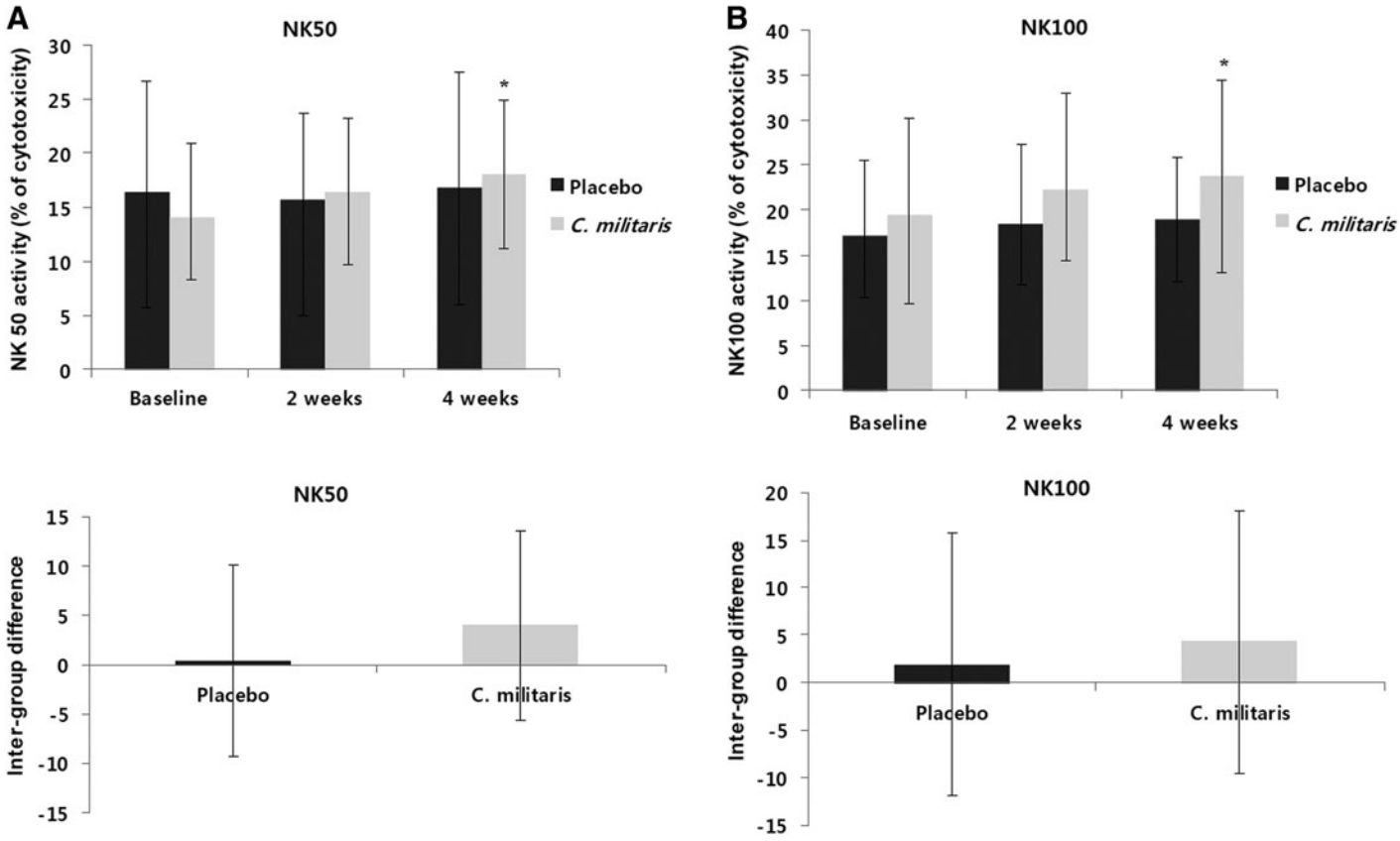

FIG. 1. All data are expressed as mean ± SD or number (percentage) of participants. **(A)** Effector to target ratio 50:1 by LDH cytotoxic assay. **(B)** Effector to target ratio 100:1 by LDH cytotoxic assay. **(C)** Effector to target ratio 200:1 by LDH cytotoxic assay. *NK50, NK100 activity showed a statistically significant increase compared with the baseline after 4 weeks of *C. militaris* administration. The NK200 activity showed a statistically significant increase after 2 and 4 weeks of *C. militaris* administration (P value $< .05$). There was no significant increase in the placebo group. †Statistically significant intergroup differences in the NK200 variation were found after 2 and 4 weeks of *C. militaris* administration compared to the placebo group (P value $< .05$). *C. militaris*, *Cordyceps militaris*; LDH, lactate dehydrogenase; NK cell, natural killer cell.

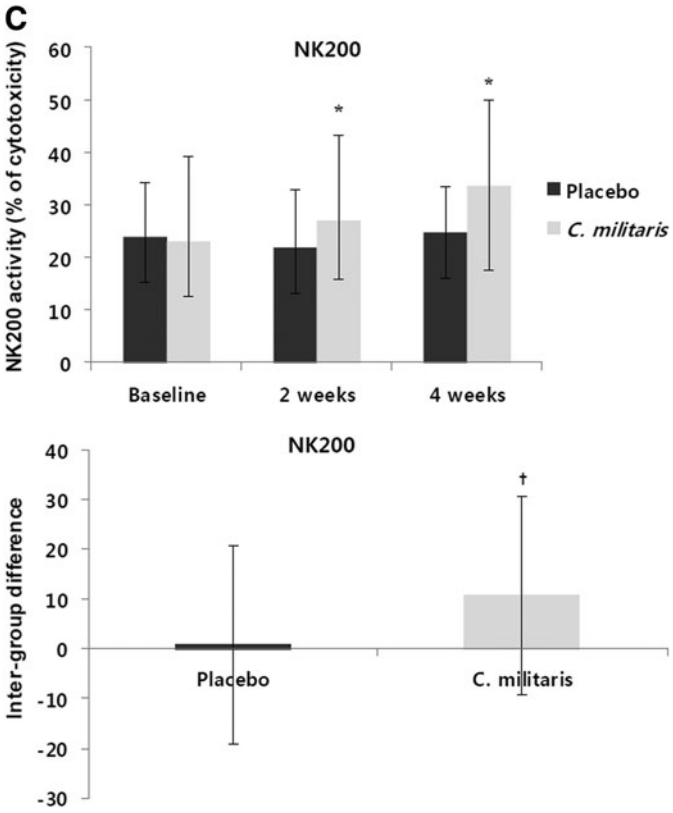

FIG. 1. (Continued).

IL-2. IL-2 increased by 217.30 ± 554.82 pg/mL, from 492.85 ± 488.62 pg/mL before administration to 710.15 ± 284.62 pg/mL after administration in the *C. militaris* group ($P = .0096$), but decreased by 38.16 ± 603.60 pg/mL, from 590.00 ± 465.40 pg/mL to 551.85 ± 323.76 pg/mL in the placebo group. Statistically significant intergroup differences in the IL-2 variation were found after 4 weeks of administration ($P = .0270$, Fig. 2).

TNF-α. TNF-α increased by 245.03 ± 947.46 pg/mL, from 755.47 ± 794.96 pg/mL to 1000.50 ± 423.84 pg/mL in the *C. militaris* group and increased by 127.83 ± 612.23 pg/mL, from 700.39 ± 438.72 pg/mL to 828.22 ± 518.37 pg/mL in the placebo group. Although TNF-α showed an increase in both groups after 4 weeks of administration, there was no significant intergroup difference in the TNF-α variation ($P = .2586$, Fig. 2).

Lymphocyte proliferation

The mean cell proliferation increased by 0.65 ± 0.50, from 0.3 ± 0.23 before administration to 0.96 ± 0.42 after 4 weeks of administration in the *C. militaris* group ($P < .0001$) and increased by 0.47 ± 0.47, from 0.33 ± 0.17 to 0.80 ± 0.40 in the placebo group ($P < .0001$). Statistically significant intergroup differences in the cell proliferation variation were found after 4 weeks of administration ($P = .0482$, Fig. 2).

Adverse effect

No case of serious adverse effects related to the administration was reported during the study. In addition, no clinically significant difference was found in clinical indexes, including vital signs and other diagnostic blood tests, between before and after administration (Table 2).

DISCUSSION

C. militaris is a medical mushroom traditionally used mainly in East Asia. Numerous studies and reports have described the effect of *Cordyceps*, including enhancement of immunity, activation of basal metabolism, and improvement of liver and renal functions.[1–7]

Cordyceps contains various chemical constituents that exert biological activities; of these, nucleotides and nucleotide derivatives, such as cordycepin, cordycepic acid, and guanosine, are some of the most important pharmacologically active compounds.[15] Animal testing has demonstrated that *C. militaris* has an anticancer effect, preventing growth of sarcoma on the basis of the increased splenic NK cell and T-cell count and the increased IL-2 generation of spleen cells.[16] In particular, cordycepin has anticancer effects when it is inserted in cancer cell DNA and RNA in place of adenine and has suppressed activation of reverse transcriptase to prevent HIV infection.[5] Antioxidative and anti-inflammatory activities of *C. militaris* include removal of free radicals, prevention of nitric oxide (NO) generation, and manifestation of inducible NO synthase.[17–20]

The best-known pharmaceutical activity of *Cordyceps* is to enhance immunity. While this has been demonstrated in animal tests, almost no testing has been conducted in human subjects. This study attempted to determine its effectiveness for enhancing immunity in healthy male adults. The existing animal testing found that *C. militaris* enhanced the NK cell activity and increased secretion of Th1 cytokines to increase spleen cell proliferation in an immunosuppressed mouse.[8] In other words, *C. militaris* was found to be more effective in enhancing cell-mediated immunity rather than humoral immunity in mice. In this study, the NK cell activity, the Th1 cytokine cluster, and cell proliferation were used as indexes in people.

Xu *et al.* observed that intraperitoneal injection of *Cordyceps* resulted in an enhanced NK activity and decreased colony formation of B16 melanoma in mice.[21] Liu *et al.* found that administration of *Cordyceps* led to a dose-dependent enhancement of NK cell activity in healthy persons and leukemia patients.[22] Likewise, this study confirmed that the administration of *C. militaris* resulted in enhanced NK cell activity. The prior study included six subjects and an animal model. However, our study included 80 subjects and human subjects.

Kim *et al.* also observed that while Th1 cytokines, including IL-2, IL-12, IFN-γ, and TNF-α, showed a remarkable increase, IL-4 and IL-10 did not, in an immunosuppressed mouse.[8] Gao *et al.* found that *Cordyceps* adjusted the balance of Th1/Th2 cytokines and, in particular, increased immune responses of Th1 cytokines in condyloma acuminatum

patients: increased IL-2 and decreased IL-10 were observed in the *Cordyceps*-administered group.[23] This study used the Th1 cytokine cluster alone as an assessment index and confirmed that *Cordyceps* administration increased some Th1 cytokines, including IL-2 and IFN-γ, as in the above-mentioned clinical studies. However, using mouse splenocytes, Jeong *et al.* found that *Cordyceps* enhanced both cellular and humoral immunity: in the *Cordyceps* administered group, IL-12 increased by 2.9 times and IL-4 and IL-10 in the Th2 cytokine cluster increased by 1.9 times and 1.8 times, respectively.[24] Therefore, conduct of additional studies of the Th2 cytokines responses is needed.

There was a finding that the administration of *Cordyceps* above a certain threshold or higher concentrations increased lymphocyte proliferation and CD marker expression in a mouse[25]; this study also confirmed that the administration of *Cordyceps* resulted in increased lymphocyte proliferation in a human model.

No serious adverse reactions to *Cordyceps* administration have been reported. While one study reported that it could cause dry mouth, nausea, loss of appetite, diarrhea, or dizziness in some patients,[15] this study found no such adverse events.

The fruit bodies of wild *C. militaris* are expensive due to host specificity and rarity in nature; they grow extremely slow in nature, their growth is restricted to specific areas, and their sizes are very small. Therefore, collection of sufficient quantities for extensive use as a drug remedy is prohibitive. Solid culture of mushrooms requires a long period of time for completion of a fruiting body, thus, many attempts have been made to obtain useful and potent cellular or extracellular substances from a brown rice culture for use in formulation of nutraceuticals and functional foods. Use of brown rice culture has potential advantages of higher mycelial production in a compact space and shorter time with less chance of contamination. We used this method to obtain a large amount of raw material of *Cordyceps*, and clinical tests were performed.

This study was conducted only in healthy male adults to minimize the effects of women's sexual hormonal changes on immunity. Veenstra *et al.* reported that a pregnant woman had activated monocytes and granulocytes and the ratio of Th1 to Th2 cytokine production in lymphocyte was lower.[26] Ansar *et al.* also suggested sexual dimorphism: women are more resistant to infection and have relatively stronger immune responses than men. As a result, women have a higher prevalence of autoimmune diseases and expression of immune-based diseases, such as multiple sclerosis, asthma, or systemic lupus erythematosis, affected by the reproductive status.[27] That is, women become more vulnerable to disease during specific periods in the menstrual cycle or during pregnancy and such vulnerability

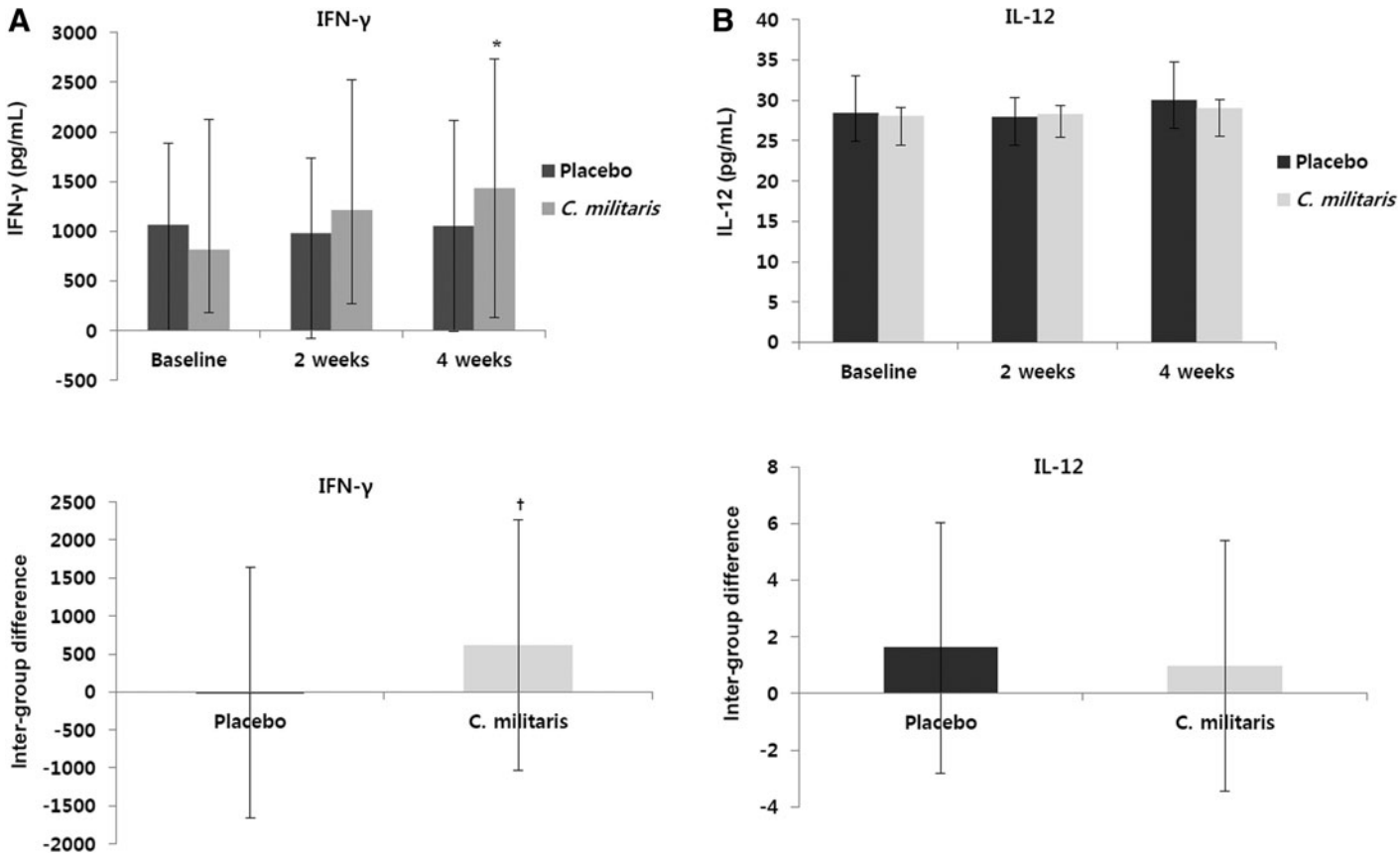

FIG. 2. (Continued).

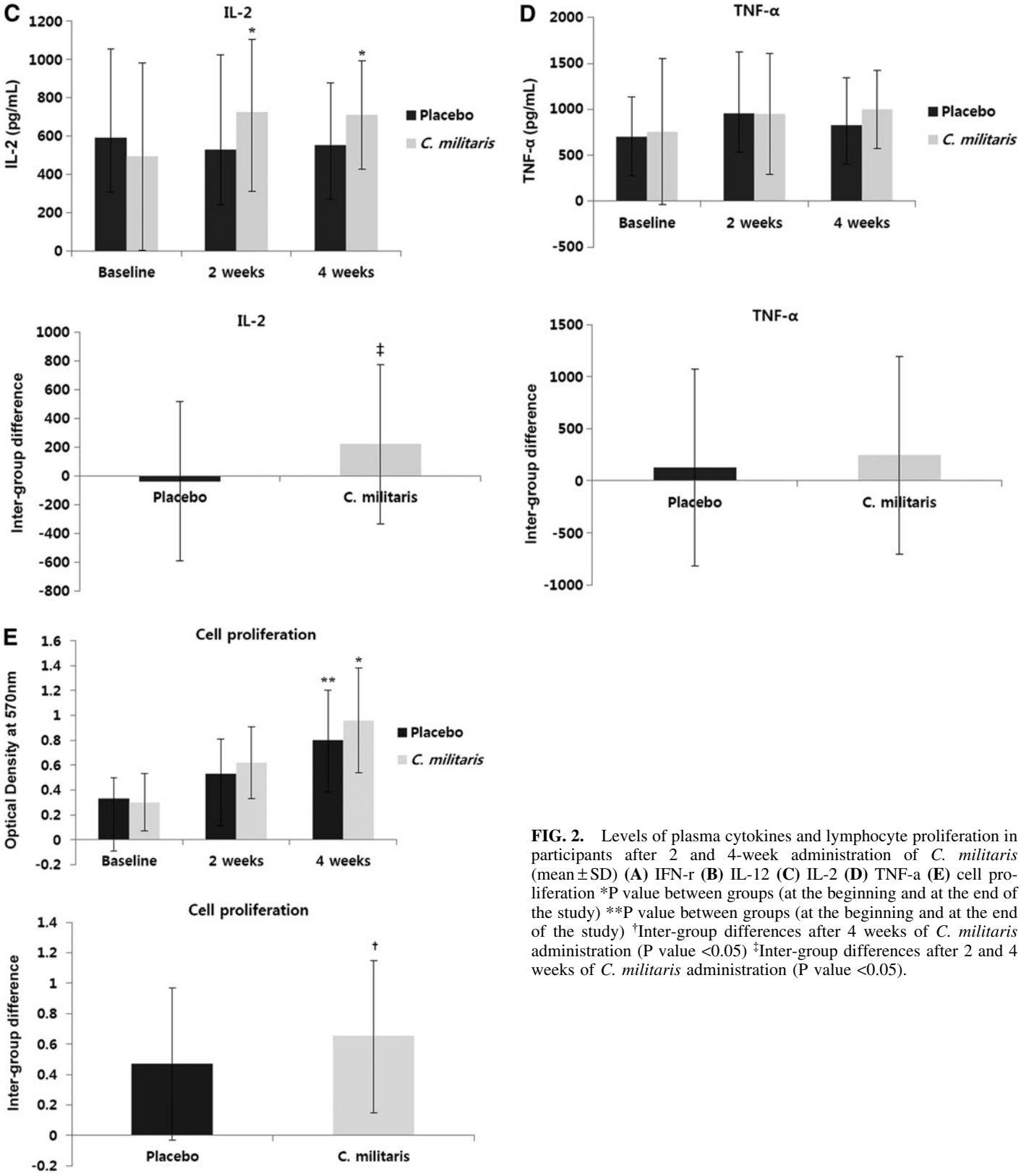

FIG. 2. Levels of plasma cytokines and lymphocyte proliferation in participants after 2 and 4-week administration of *C. militaris* (mean ± SD) **(A)** IFN-r **(B)** IL-12 **(C)** IL-2 **(D)** TNF-a **(E)** cell proliferation *P value between groups (at the beginning and at the end of the study) **P value between groups (at the beginning and at the end of the study) †Inter-group differences after 4 weeks of *C. militaris* administration (P value <0.05) ‡Inter-group differences after 2 and 4 weeks of *C. militaris* administration (P value <0.05).

Table 2. Changes in Vital Signs and Laboratory Tests of Two Groups

	Placebo group (n=40)	C. militaris *group (n=39)*	P *value*
Vital signs difference			
Body temperature (°C)	0.11 ± 0.62	−0.09 ± 0.58	.0679
SBP (mmHg)	−2.41 ± 6.51	−2.15 ± 6.13	.4292
DBP (mmHg)	−1.95 ± 7.55	−2.41 ± 7.91	.3964
Pulse rate (beat/min)	−3.46 ± 7.08	−2.46 ± 7.98	.2800
Laboratory tests difference			
White blood cell ($\times 10^3/mm^3$)	0.01 ± 1.03	0.04 ± 1.64	.4672
Hemoglobin (g/dL)	3.53 ± 21.48	0.22 ± 0.59	.1709
Hematocrit (%)	2.55 ± 8.10	8.21 ± 59.14	.2788
Platelet ($\times 10^3/mm^3$)	−2.26 ± 46.78	0.18 ± 21.75	.3843
Aspartate aminotransferase (IU/L)	8.37 ± 49.24	−0.44 ± 6.03	.1373
Alanine aminotransferase (IU/L)	10.86 ± 67.28	−0.90 ± 7.72	.1426
Alkaline phosphatase (IU/L)	6.54 ± 34.00	5.10 ± 28.18	.4198
γ-Glutamyl transferase (IU/L)	−4.28 ± 10.87	−3.08 ± 9.35	.3006
Blood urea nitrogen (mg/dL)	1.09 ± 4.33	0.15 ± 3.52	.1477
Creatinine (mg/dL)	−0.01 ± 0.09	−0.01 ± 0.08	.4945
Lactate dehydrogenase (IU/L)	33.67 ± 78.89	−29.06 ± 501.91	.2227

All data are expressed as mean ± SD or number (percentage) of participants. There was no significant difference between two groups after 4 weeks of administration of *C. militaris* compared to the placebo group.

P values by *t*-test.

during pregnancy is due to the lowered Th1/Th2 cytokine ratio. Accordingly, because immunity can depend on women's sexual hormonal changes, this study excluded women to avoid the effects of the hormonal changes due to the menstrual cycle, oral contraceptive use, or the postmenopausal state. Therefore, further research considering women's sexual hormone is needed.

Lowered immunity is regarded as an important cause of decline in the functions of the human body and in increasing morbidity of diverse types of viral and bacterial infection, malignancy, or organ failure as one gets older. Limited phenotypic and functional changes in the T-cell component of adaptive immunity are also found among very healthy elderly people.[28,29] One study reported that the elderly are not only more sensitive to influenza virus infection but also benefit less from influenza virus vaccination.[30] In this study, since the statistical analysis in participants aged 50 or older generated results that were not significantly different from all age groups, *Cordyceps* is also expected to be effective for enhancing immunity in the elderly population.

Cordyceps is also expected to be useful for immunocompromised patients with malignancy, chemotherapy or radiation therapy, viral infection, such as AIDS, influenza, or chronic viral hepatitis, or bacterial infection, long-term steroid therapy, or chronic dysfunctions of organ systems, such as congestive heart failure, chronic kidney disease, chronic obstructive pulmonary disease, or diabetes mellitus, as well as for those whose immunity has been lowered naturally. Since *C. militaris* was found to be effective for enhancing immunity in an immunosuppressed mouse, conduct of a clinical trial is necessary to determine its validity and safety in immunocompromised patients.

Despite other limitations, including the relatively short period of research and exclusion of women, it is expected that *C. militaris* extract can be used effectively and safely to enhance immunity since no clinically significant adverse effects were found during the experiment.

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AUTHOR DISCLOSURE STATEMENT

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