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Original Article

Regulation of human cytokines by *Cordyceps militaris*



Yong Sun^{a,b}, Yani Shao^a, Zhiguo Zhang^c, Lianfen Wang^b,
Alfred M. Mariga^{a,d}, Guangchang Pang^e, Chaoyu Geng^e, Chi-Tang Ho^f,
Qihui Hu^a, Liyan Zhao^{a,*}

^a College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China^b Beijing Food Research Institute, Beijing 100162, China^c College of Food and Bioengineering, Shandong Polytechnic University, Jinan 250353, China^d Department of Dairy and Food Science and Technology, Egerton University, Egerton 536, Kenya^e College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China^f Department of Food Science, Rutgers University, New Brunswick, NJ 08901, USA

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ABSTRACT

Cordyceps (*Cordyceps militaris*) exhibits many biological activities including antioxidant, inhibition of inflammation, cancer prevention, hypoglycemic, and antiaging properties, etc. However, a majority of studies involving *C. militaris* have focused only on *in vitro* and animal models, and there is a lack of direct translation and application of study results to clinical practice (e.g., health benefits). In this study, we investigated the regulatory effects of *C. militaris* micron powder (3 doses) on the human immune system. The study results showed that administration of *C. militaris* at various dosages reduced the activity of cytokines such as eotaxin, fibroblast growth factor-2, GRO, and monocyte chemoattractant protein-1. In addition, there was a significant decrease in the activity of various cytokines, including GRO, sCD40L, and tumor necrosis factor- α , and a significant downregulation of interleukin-12(p70), interferon- γ inducible protein 10, and macrophage inflammatory protein-1 β activities, indicating that *C. militaris* at all three dosages downregulated the activity of cytokines, especially inflammatory cytokines and chemokines. Different dosages of *C. militaris* produced different changes in cytokines.

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1. Introduction

Cordyceps militaris, also known as north *Cordyceps sinensis*, is the most common species of the genus *Cordyceps* (Division:

Ascomycotina; Order: Hypocreales; Family: Clavicipitaceae) [1]. The bioactive components in *C. militaris* such as cordycepin, cordyceps polysaccharide, cordycepic acid, and superoxide dismutase [2] have been reported to possess various biological properties, including antioxidant [3],

* Corresponding author. College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China.

E-mail address: zhlychen@njau.edu.cn (L. Zhao).

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antineoplastic [4], antiaging [5], hypoglycemic [6], and inhibition of inflammation [7]. Although many studies have documented the biological activity of *C. militaris*, most of these have focused on *in vitro* or animal models, which are unable to reveal the impact of these components on immune regulation because of the cutoff of communication between immune cells.

In elucidating the action mechanism of the phytochemicals in *C. militaris*, we conducted a human study to gain a better understanding of regulatory effects of this special *Cordyceps* species. Twelve young volunteers were selected as participants for oral administration of *C. militaris* submicron powder. A liquid chip scanner was used to determine the content changes of 39 cytokines and to explore regulatory functions and signal pathways of *C. militaris* with respect to the immune system; in addition we also evaluated its antitumor activity. A cell communication network of the human immune system was established based on the experimental data.

2. Methods

2.1. Materials

C. militaris was purchased from Yancheng Shennong Functional Food Co., Ltd., dried, and crushed into a powder form with a particle size smaller than 48 μm in diameter. A 3K15 high-speed refrigerated centrifuge was purchased from Sigma Co. (St. Louis, MO, USA). A light biochemical incubator was purchased from Donglian Ltd. Co. (Harbin, China). The human kit with 96 enzyme-labeled plates and the liquid chip scanner were purchased from Millipore Corporation (Billerica, MA, USA).

2.2. Participants

Volunteers for the study were five male and seven female healthy college students aged from 20 years to 21 years; the average male and female body weights were 65 ± 2 kg and 48 ± 2 kg, respectively. The body mass index of male and female volunteers was in the range of 21.14–21.46 and 17.96–18.36, respectively. All volunteers signed informed, responsibility, and safety agreements and were randomly divided into three dosage groups of four students. All experiments were conducted according to the Declaration of Helsinki and all experimental protocols were approved by the ethical committee for human trials of the College of Biotechnology and Food Science, Tianjin University of Commerce (Tianjin, China). The volunteers were required to follow a schedule of regular and normal hours of work and rest, as well as strict restrictions on strenuous exercise, cigarettes smoking, or alcohol. Except for nonfunctional meals provided for these volunteers, any other functional foods, drugs, or beverages were prohibited. During the study period, all volunteers stayed in a relaxed environment to protect them from sources of stress [8].

During the 2-day period of experimental duration, the volunteers were provided with unbiased meals on the 1st day and with the same meals with the addition of the test foods on

the 2nd day. Blood samples were collected at 2 PM every day, incubated at 4 °C for 16 hours, centrifuged at 3200g for 10 minutes, and stored at –70 °C for future use. Test results from the 1st day were used as the control.

2.3. Administration dosages and methods

In this study, the submicron powder at high, medium, and low dosages (3, 1.5, and 0.5 g) was mixed with water to form a paste, respectively. The paste was steamed for 15 minutes before oral administration to the volunteers.

2.4. Cytokine detection

A Millipore human kit with 96-well enzyme-labeled plates was used to detect the following cytokines: epidermal growth factor (EGF), eotaxin, fibroblast growth factor-2 (FGF-2), Flt-3L, fractalkine, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), growth regulated oncogene (GRO), interferon- $\alpha 2$ (IFN- $\alpha 2$), IFN- γ , interleukin-1 α (IL-1 α), IL-1 β , IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, IFN- γ inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), MCP-3, macrophage-derived chemokine (MDC), macrophage inflammatory protein-1 α (MIP-1 α), MIP-1 β , sCD40L, sIL-2Ra, transforming growth factor- α (TGF- α), tumor necrosis factor- α (TNF- α), TNF- β , and vascular endothelial growth factor (VEGF). The content-change rates of these cytokines can be expressed using following equation:

$$\text{Change rate(\%)} = (C_{\text{Experimental}} - C_{\text{Control}}) / C_{\text{Control}} \quad [1]$$

2.5. Data analysis

SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of experimental results using t test. Significant differences or extreme significant differences were considered at $p < 0.05$ and $p < 0.01$, respectively.

3. Results

3.1. Impact of low dosage of *C. militaris* on cytokines

Following the administration of *C. militaris* at the 0.5 g dosage, no obvious changes were observed in 19 cytokines, including IL-1 α , IL-1 β , IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-12(p40), IL-13, IL-15, IL-17, MCP-3, MIP-1 α , sIL-2Ra, and TNF- β , whereas the concentrations of IFN- γ in blood samples taken from the volunteers increased from the 1st day to the 2nd day, which suggested upregulation of IFN- γ . By contrast, the concentrations of EGF, eotaxin, FGF-2, Flt-3L, fractalkine, G-CSF, GM-CSF, GRO, IFN- $\alpha 2$, IL-8, IL-10, IL-12(p70), IP-10, MCP-1, MDC, MIP-1 β , sCD40L, TGF- α , TNF- α , and VEGF in blood samples from the volunteers decreased from the 1st day to the 2nd day, which suggested that these cytokines were down-regulated (Fig. 1).

Results of the t test revealed a significant decrease in eotaxin, FGF-2, GRO, and MCP-1, suggesting that *C. militaris* at a

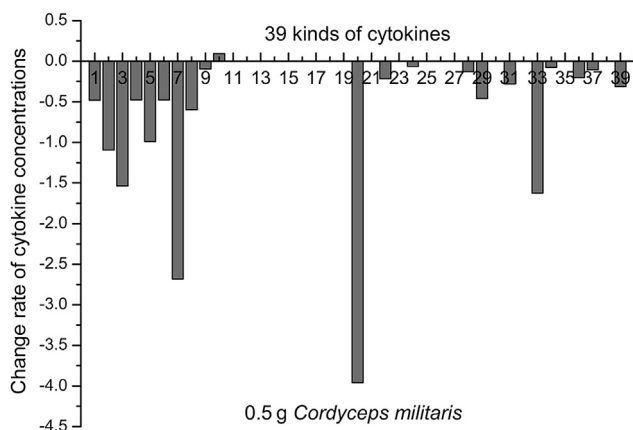


Fig. 1 – Administration of *Cordyceps militaris* at the 0.5 g dosage and the rate of change in the concentration of different cytokines. 1. Epidermal growth factor; 2. Eotaxin; 3. Fibroblast growth factor-2; 4. Flt-3L; 5. Fractalkine; 6. Granulocyte-colony stimulating factor; 7. Granulocyte-macrophage colony-stimulating factor; 8. GRO; 9. Interferon- α 2 (IFN- α 2); 10. IFN- γ ; 11. Interleukin-1 α (IL-1 α); 12. IL-1 β ; 13. IL-1ra; 14. IL-2; 15. IL-3; 16. IL-4; 17. IL-5; 18. IL-6; 19. IL-7; 20. IL-8; 21. IL-9; 22. IL-10; 23. IL-12(p40); 24. IL-12(p70); 25. IL-13; 26. IL-15; 27. IL-17; 28. IFN- γ inducible protein 10; 29. Monocyte chemoattractant protein-1 (MCP-1); 30. MCP-3; 31. Macrophage-derived chemokine; 32. Macrophage inflammatory protein-1 α (MIP-1 α); 33. MIP-1 β ; 34. sCD40L; 35. sIL-2Ra; 36. Transforming growth factor- α ; 37. Tumor necrosis factor- α (TNF- α); 38. TNF- β ; and 39. Vascular endothelial growth factor. Similarly hereinafter.

dosage of 0.5 g can downregulate these four cytokines dramatically (Table 1).

3.2. Impact of medium dosages of *C. militaris* on cytokines

The administration of *C. militaris* at a dosage of 1.5 g produced no observable changes in Flt-3L, IL-1 β , IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-7, IL-9, IL-12(p70), IL-13, IL-15, IL-17, IP-10, MDC, or TNF- β , whereas the concentrations of FGF-2, IL-10, IL-12(p40), MCP-3, sIL-2Ra, and VEGF in blood samples from the volunteers were much higher on the 2nd day, suggesting that these cytokines were upregulated (Fig. 2). In addition, the concentrations of

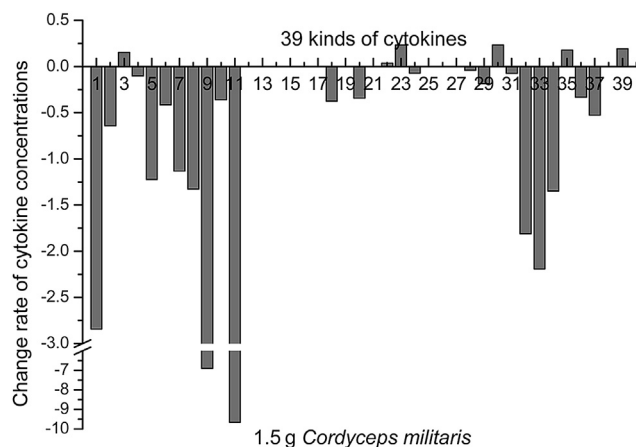


Fig. 2 – Administration of *Cordyceps militaris* at the 1.5 g dosage and the rate of change in the concentration of different cytokines.

EGF, eotaxin, fractalkine, G-CSF, GM-CSF, GRO, IFN- α 2, IFN- γ , IL-1 α , IL-6, IL-8, MCP-1, MIP-1 α , MIP-1 β , sCD40L, TGF- α , and TNF- α in blood samples from the volunteers decreased on the 2nd day, suggesting that these cytokines were downregulated.

Results of the t test revealed a significant decrease in GRO, sCD40L, and TNF- α levels, which indicated that *C. militaris* at a dosage of 1.5 g can downregulate these three cytokines (Table 2).

3.3. Impact of high dosages of *C. militaris* on cytokines

The administration of *C. militaris* at a dosage of 3.0 g did not result in obvious changes in Flt-3L, IFN- γ , IL-1 α , IL-1 β , IL-1ra, IL-3, IL-5, IL-6, IL-7, IL-9, IL-12(p40), IL-13, IL-17, MDC, MIP-1 α , sIL-2Ra, or TNF- β , whereas the concentrations of IL-4, IL-8, and IL-15 in blood samples from the volunteers were much higher on the 2nd day compared with that of the 1st day, suggesting that these cytokines were upregulated (Fig. 3). However, the concentrations of EGF, eotaxin, FGF-2, fractalkine, G-CSF, GM-CSF, GRO, IFN- α 2, IL-2, IL-10, IL-12(p70), IP-10, MCP-1, MCP-3, MIP-1 β , sCD40L, TGF- α , TNF- α , and VEGF in blood samples from the volunteers were lower on the 2nd day, which suggested that these cytokines were downregulated.

Results of the t test revealed a significant decrease in IL-12(p70), IP-10, MCP-1, and MIP-1 β levels, indicating that *C.*

Table 1 – Cytokines exhibiting significant changes after *Cordyceps militaris* administration at a dosage of 0.5 g.

Cytokines	$C_{Control}$ /(pg/mL)				$C_{Experimental}$ /(pg/mL)				Average change rate/%	p
Eotaxin	52.3	55.7	67.8	75.5	42.6	18.6	24.2	55.7	-1.095*	0.052↓
FGF-2	34.9	3.8	36.5	15.3	8.0	3.49	17.3	5.9	-1.538*	0.095↓
GRO	428	355	149	438	409	210	66.7	309	-0.597**	0.045↓
MCP-1	105	100	155	226	78.6	61.1	111	154	-0.459**	0.018↓

*p < 0.05, significant difference.

**p < 0.01, extreme significant difference.

FGF-2 = fibroblast growth factor-2; MCP-1 = monocyte chemoattractant protein-1; ↓ = decrease in the mean concentration. Similarly hereinafter.

Table 2 – Cytokines exhibiting significant changes after *Cordyceps militaris* administration at a dosage of 1.5 g.

Cytokines	$C_{Control}/(pg/mL)$					$C_{Experimental}/(pg/mL)$			Average change rate/%	p
	519	483	340	340	105	153	156	232		
GRO	519	483	340	340	105	153	156	232	-1.327*	0.022 ↓
sCD40L	24,017	24,017	24,017	18,756	5454	24,017	14,456	8070	-1.347**	0.084 ↓
TNF- α	3.7	4.4	8.4	2.2	1.8	3.9	5.8	1.5	-0.525**	0.065 ↓

TNF- α = tumor necrosis factor- α .

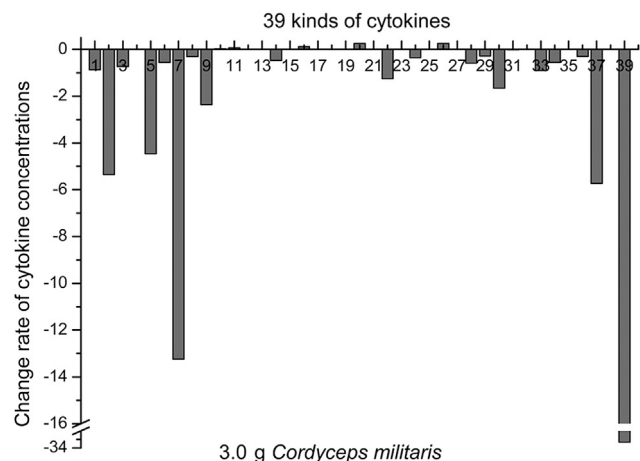


Fig. 3 – Administration of *Cordyceps militaris* at the 3.0 g dosage and the rate of change in the concentration of different cytokines.

militaris at high dosages can downregulate these four cytokines (Table 3).

4. Discussion

In this study, the effects of administering *C. militaris* at low, medium, and high dosages (0.5 g, 1.5 g, and 3.0 g, respectively) were analyzed. Downregulation by *C. militaris* was observed for different chemokines at all three dosages, including eotaxin, FGF-2, GRO, and MCP-1 at low dosage; GRO, sCD40L, and TNF- α at medium dosage; and IL-12(p70), IP-10, MCP-1, and MIP-1 β at high dosage. This regulatory effect is important because chemokines play important physiological and pathological roles in many diseases, including inflammation, cancer, cancer metastasis, autoimmune diseases, hypersensitivity, acquired immunodeficiency syndrome, and other diseases associated with the involvement of chemokines and

their receptors [9–14]. *C. militaris* at low and medium dosages was observed to downregulate the expression of GRO, which can promote the growth of epithelial cells and endothelial cells [15], whereas at the medium dosage *C. militaris* exerted a major influence on TNFs, such as sCD40L and TNF- α . TNF- α can bind to a specific receptor on the surface of the cell membrane to induce growth, differentiation, apoptosis, and inflammation [16]. At higher dosages, *C. militaris* significantly decreased the levels of IP-10 and MIP-1 β . The concentration of IP-10, which originates in monocytes/macrophages and T cells, has been observed to increase in the neural system, liver, and spleen as an inflammatory response to virus infection, which is a consequence of immune response to pathogen invasion. However, recent studies have reported other factors that may contribute to excessive inflammatory responses and chemostatic effects, including diet and nutrition, which may lead to transplant rejection [11]. The chemokine MIP-1 β has been found to regulate the migration of dendritic cells. A previous study demonstrated that increased and decreased expressions of MIP-1 β were related to the early and late stages of colon cancer, respectively [17]. The administration of *C. militaris* at both low and high dosages was also observed to downregulate cytokine MCP-1. MCP-1 is the initial factor associated with inflammation, and can form a cascade reaction or mediate excessive inflammatory response by inducing and regulating other inflammatory factors [18]. MCP-1 can also boost the growth, survival, and migration of tumor cells [19]. Results of this study indicate that *C. militaris* can exhibit antitumor functions by downregulating the activity of these cytokines.

In conclusion, we have demonstrated that the consumption of *C. militaris* at three dosages can significantly reduce the concentrations of cytokines, resulting in downregulation of the immune system in the body. Different dosages of *C. militaris* produced significant changes in different cytokines and in cell communication. The strongest immunoregulatory role of *C. militaris* appeared to occur with administration of the medium dosage. These results suggest that *C. militaris* can downregulate the immune system by reducing inflammatory

Table 3 – Cytokines exhibiting significant changes after *Cordyceps militaris* administration at a dosage of 3.0 g.

Cytokines	$C_{Control}/(pg/mL)$					$C_{Experimental}/(pg/mL)$			Average change rate/%	p
	1.7	1.1	2.2	1.9	1	0.92	1.7	1.5		
IL-12(p70)	1.7	1.1	2.2	1.9	1	0.92	1.7	1.5	-0.364*	0.026 ↓
IP-10	502	497	358	575	435	180	298	450	-0.599**	0.099 ↓
MCP-1	127	101	80.7	129	87.9	91.4	70.2	87.6	-0.293**	0.064 ↓
MIP-1 β	43.5	40.3	25.9	5.7	26.9	21.4	23.9	1.86	-0.912**	0.097 ↓

IL-12(p70) = interleukin-12(p70); IP-10 = interferon- γ inducible protein 10; MCP-1 = monocyte chemoattractant protein-1; MIP-1 β = macrophage inflammatory protein-1 β .

chemokines such as IP-10, MCP-1, and eotaxin, which can in turn induce the migration of immune cells including macrophages and monocytes to the infection locations under inflammatory environments as well as by stimulating the secretion of TNF- α and by the activation of nuclear factor- κ B. However, the significant reduction of TNF- α also suggests decreased activity of macrophages and monocytes. All of these results indicate that *C. militaris* at the various dosages can reduce the excessive immune responses, which is expected to produce beneficial effects against some diseases resulting from appropriate diet or autoimmunity, including an inhibitory role in tumorigenesis.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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