Dietary Wolfberry Supplementation Enhances the Protective Effect of Flu Vaccine against Influenza Challenge in Aged Mice[1,2]

Xiaogang Du,[3,4] Junpeng Wang,[3,4] Xinli Niu,[4] Donald Smith,[5] Dayong Wu,[4]* and Simin Nikbin Meydani[4]*

[4]Nutritional Immunology Laboratory, and [5]Comparative Biology Unit, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA

Abstract

Current vaccines for influenza do not fully protect the aged against influenza infection. Although wolfberry (goji berry) has been shown to improve immune response, including enhanced antibody production, after vaccination in the aged, it is not known if this effect would translate to better protection after influenza infection, nor is its underlying mechanism well understood. To address these issues, we conducted a study using a 2 × 2 design in which aged male mice (20–22 mo) were fed a control or a 5% wolfberry diet for 30 d, then immunized with an influenza vaccine or saline (control) on days 31 and 52 of the dietary intervention, and finally challenged with influenza A/Puerto Rico/8/34 virus. Mice fed wolfberry had higher influenza antibody titers and improved symptoms (less postinfection weight loss) compared with the mice treated by vaccine alone. Furthermore, an in vitro mechanistic study showed that wolfberry supplementation enhanced maturation and activity of antigen-presenting dendritic cells (DCs) in aged mice, as indicated by phenotypic change in expression of DC activation markers major histocompatibility complex class II, cluster of differentiation (CD) 40, CD80, and CD86, and functional change in DC production of cytokines interleukin-12 and tumor necrosis factor-α as well as DC endocytosis. Also, adoptive transfer of wolfberry-treated bone marrow DCs (loaded with ovalbumin$_{323–339}$-peptide) promoted antigen-specific T cell proliferation as well as interleukin-4 and interferon-γ production in CD4$^+$ T cells. In summary, our data indicate that dietary wolfberry enhances the efficacy of influenza vaccination, resulting in better host protection to prevent subsequent influenza infection; this effect may be partly attributed to improved DC function. *J. Nutr.* doi: 10.3945/jn.113.183566.

Introduction

Aging is associated with increased incidence and severity of infectious diseases (1–4). Among these diseases, influenza infection is a significant health problem worldwide. Approximately 500,000 people die annually from influenza infection (5,6). In the United States, seasonal influenza infection causes ~200,000 cases of hospitalization and 36,000 deaths (7). It is well documented that influenza-associated morbidity and mortality are much higher in the elderly. Approximately 90% of the mortality related to influenza occurs in those aged ≥65 y (7), which is mainly attributed to an age-related progressive decline in the immune response, or immunosenescence, as it is commonly referred to (8–10). Vaccination is currently the most efficient strategy to prevent influenza infection; however, vaccine efficacy is significantly diminished in the elderly due to the age-related impairment of both innate and adaptive immune responses (11–16). Thus, developing strategies to enhance vaccine effectiveness could help reduce the high morbidity and mortality associated with influenza infection in the elderly.

Wolfberry (*Lycium barbarum*, Solanaceae), or goji berry, has traditionally been used in Chinese herbal medicine. Recent research also suggests a number of potential health benefits including antioxidant and antitumor activities (17,18) as well as neuroprotective (19) and immunoenhancing effects (20–25). Of particular note, a milk-based preparation of wolfberry has been shown to improve cell-mediated immune response in aged mice (24) and to elevate antibody titers after influenza vaccination in elderly persons (25). However, the impact of wolfberry on improving vaccine efficacy against a new infection has not been studied. Because this question cannot be addressed in humans, we used an influenza infection animal model to determine whether wolfberry can potentiate a vaccine's efficacy in protecting the host when infected by influenza. We further conducted a

mechanistic study to determine whether wolfberry's effect is mediated through enhancing maturation and antigen-presenting function of dendritic cells (DCs)[6], a key step in producing effective antibody response.

Materials and Methods

Animals. Young (4–6 mo) and aged (20–22 mo) specific pathogen–free male C57BL/6 mice were obtained from the National Institute on Aging colonies at Charles River Laboratories. Aged mice were used to determine wolfberry's effect on DC function in the in vitro study and on vaccine efficacy in the dietary supplementation study. Young mice were used to obtain T cells to determine the effect of the adoptively transferred aged DCs (with and without wolfberry supplementation) on T cell response. Mice were kept at a constant temperature and humidity with a 12-h light-dark cycle. Mice were housed individually in cages and consumed water and food ad libitum. At the end of the study, mice were killed by CO_2 asphyxiation followed by exsanguination. All procedures of handling the animals were performed in accordance with the protocols approved by the Animal Care and Use Committee of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University and conducted according to the NIH Guidelines for the Care and Use of Laboratory Animals.

Dietary supplementation. Aged mice were randomly divided into 4 groups (n = 13/group). Two groups were each fed a standard AIN-93M diet (26) supplemented with a 5% milk-based preparation of wolfberry (Nestec) or with 5% corn starch (control) during the entire experimental period. The milk-based preparation of wolfberry contained 530 mg/g of wolfberry fruit, 290 mg/g of skimmed milk, and 180 mg/g of maltodextrin.

Immunization and infection. The influenza vaccine was prepared by mixing the influenza A hemagglutinin protein derived from A/Puerto Rico/8/34 (Sino Biological) with an aluminum adjuvant (Thermo Fisher Scientific) at a ratio of 1:1. Mice were immunized with the influenza vaccine by i.p. injection at day 31 of wolfberry supplementation and received a booster at day 52. Blood was collected at day 66 for antibody titer analysis. Half of the mice in each diet group were immunized, and the other half were given saline to serve as a control. At day 73, mice were infected with influenza A/Puerto Rico/8/34 virus (provided by Barry Ritz, Drexel University) as previously described (23). After infection, mice were monitored daily for weight loss and mortality.

Hemagglutination inhibition assay. Serum (10 μL) was pretreated with receptor-destroying enzyme (Denka Seiken) and then serially diluted (1:2) in V-bottom microtiter plates. Twenty-five microliters of solution containing 4 hemagglutination units of influenza virus A/PR/8/34 were added to each well and incubated at 37°C for 1 h. Thereafter, 25 μL of 1% turkey red blood cells (Lampire Biologicals) in PBS were added to each well, and the plates were incubated at room temperature for 30 min. The hemagglutination inhibition titer was defined as the reciprocal dilution of the last well that showed inhibition of hemagglutination.

Antibody titer. Anti-influenza hemagglutination-specific antibodies in serum were measured by ELISA following a protocol that has been previously described (27). The purified hemagglutination protein (2 mg/L) was used to coat ELISA plates. The immunoglobulin G (IgG) antibody titers were defined as the highest dilution giving a ratio >2:1 between serum and the negative control.

DC generation. A group of nontreated aged mice were killed, and bone marrow (BM) cells were collected by flushing the femurs and tibias with

RPMI 1640 medium (BioWhittaker) containing 10% FBS (Gibco Invitrogen). The cells were then filtered through a 40-μm cell strainer (BD Falcon) and cultured in RPMI 1640/10% FBS in the presence of murine recombinant granulocyte-macrophage colony-stimulating factor at 20 μg/L and murine recombinant interleukin (IL)-4 at 20 μg/L (both from R&D Systems). At day 2, two-thirds of the medium were replaced with fresh media. At day 5, floating and loosely adhering cells were harvested and seeded into 24-well plates as BM-derived DCs.

In vitro treatment of BM DCs with wolfberry extract. BM DCs were supplemented with 0, 100, 200, 400, or 800 mg/L of a wolfberry extract (Shengyuan Biotechnology) for 48 h. At day 6, a separate set of cells was stimulated with 100 μg/L of LPS as a positive control for 24 h. At the end of the stimulation, culture medium and BM DCs were separately collected for further analyses. The endotoxin contamination in this wolfberry extract was 22.9 endotoxin units/mg. Thus, the above wolfberry solutions (100–800 μg/mL) would contain 2.29–18.3 endotoxin units of endotoxin/mL, equivalent to 2.29–18.3 pg/mL LPS of the type used in this study (*Escherichia coli* 0111:B4). LPS at 20 pg/mL had no effect on DC activation on the basis of our tests (data not shown).

Flow cytometry analysis. To measure the maturation and activation markers for DCs, BM DCs were stained with fluorescein isothiocyanate (FITC)–conjugated anti-mouse cluster of differentiation (CD) 11c, allophycocyanin-conjugated anti-mouse CD40, phycoerythrin-conjugated anti-mouse CD80, CD86, and anti-I-A [major histocompatibility complex class II (MHC-II)] or the appropriate isotype control IgG. All anti-mouse antibodies were purchased from eBioscience. Stained cells were analyzed in a BD Accuri C6 flow cytometer, and acquired data were analyzed with FlowJo7.6 software (Tree Star).

Endocytosis assay. Seven days after the start of maturation and activation processes, DCs were harvested and pulsed with 1 g/L of FITC-conjugated dextran (42,000 Da; Sigma) for 1 h at 37°C or 4°C (control). Cells were then washed 3 times with cold PBS containing 2% FBS and stained with phycoerythrin-conjugated anti-mouse CD11c. FITC-dextran uptake by DCs was analyzed by using flow cytometry.

Cytokine production. Cell-free culture supernatant was collected after DC maturation, and IL-12p40 and tumor necrosis factor-α (TNF-α) were quantified by using sandwich ELISA following the manufacturer's instructions. All reagents were from BD PharMingen.

Adoptive transfer. BM DCs (1 $\times$ 10^9/L) treated with wolfberry extract or LPS were pulsed with ovalbumin (OVA)$_{323–339}$ (100 mg/L, synthesized by the Tufts University Core Facility Laboratory) for 2 h. After washing, 5 $\times$ 10^5 cells were injected into each young C57/B6 mouse via tail vein. Mice were divided into 4 groups to receive the following: 1) wolfberry-treated DCs without OVA, 2) control DCs with OVA, 3) wolfberry-treated DCs with OVA, or 4) LPS-treated DCs with OVA. After 1 wk, mice were killed and splenocytes were isolated. Splenocytes were stimulated with 1, 10, or 100 mg/L of OVA$_{323–339}$ for 72 h. T cell proliferation response was determined by [^{3}H]-thymidine incorporation, and results are presented as counts per minute as previously described (23). Intracellular concentrations of interferon-γ (IFN-γ) and IL-4 in CD4$^+$ T cells were determined by flow cytometry.

Statistical analysis. All results are expressed as means $\pm$ SEMs. Statistical analysis was conducted by using SYSTAT statistical software (SYSTAT 12.0). One-factor ANOVA together with Fisher's least significance difference as post hoc test was used to analyze the data in the in vitro DC study to determine wolfberry's effect. Two-factor ANOVA was used to analyze data in the in vivo study to determine the effects of vaccine, diet, and vaccine $\times$ diet interaction. When 2-factor ANOVA showed significant interaction between vaccine and diet, diet effects in vaccinated and nonvaccinated groups were further determined by using t tests. A logarithmic transformation was applied to the data prior to formal analysis to reduce heterogeneity. Repeated-measures ANOVA was used for analysis of weight-loss data. Survival results were

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[6] Abbreviations used: BM, bone marrow; CD, cluster of differentiation; DC, dendritic cell; FITC, fluorescein isothiocyanate; IFN-γ, interferon-γ; IgG, immunoglobulin G; MHC-II, major histocompatibility complex class II; OVA, ovalbumin; Th, T helper.
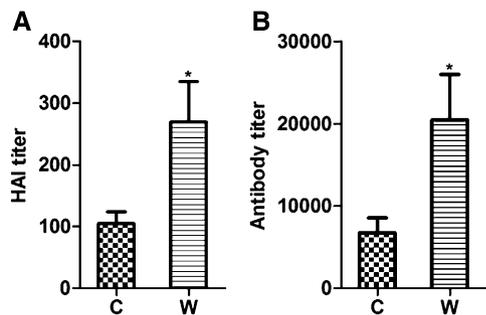
FIGURE 1 Dietary wolfberry (W) potentiated the antibody response induced by influenza vaccine in C57BL/6 mice. Blood was collected 2 wk after the second immunization, and serum was used to determine specific influenza antibody concentrations as hemagglutination inhibition (HAI) (*A*) and immunoglobulin G (*B*) titers. Values are means ± SEMs, $n = 13$. *$P < 0.05$ compared with control (C) by t test.

analyzed by using the LIFETEST procedure of the SAS System, version 9.1 (SAS Institute). Significance was set at $P < 0.05$.

Results

Dietary wolfberry potentiates the antibody response induced by influenza vaccine. As shown in **Fig. 1**, there was an overall vaccine effect ($P < 0.001$), a diet effect ($P < 0.05$), and a significant interaction between vaccine and diet effects on hemagglutination inhibition and IgG titers. These results indicate that the influenza vaccine successfully induced the host's antibody response and, further, that consumption of wolfberry might have potentiated the vaccine's efficacy.

Dietary wolfberry potentiates the efficacy of flu vaccine in reducing weight loss due to influenza virus. Weight loss is an important indicator for measuring the severity of illness in influenza-infected mice. All animals exhibited weight loss over the time course of infection (days 1 to 7 postinfection), and there were significant overall diet ($P < 0.001$) and vaccine ($P < 0.001$) effects, as well as a significant vaccine × diet interaction ($P < 0.001$) (**Fig. 2**). These results indicate that influenza vaccine reduced infection-induced weight loss, and this protective effect of vaccination was further enhanced by wolfberry supplementation. Vaccinated mice, regardless of their diet, had significantly higher survival rates ($P < 0.001$) compared with non-vaccinated mice after infection (data not shown).

Wolfberry promotes phenotypic maturation of DCs. As shown in **Table 1**, in vitro supplementation with wolfberry extract dose-dependently increased not only the percentages of DCs expressing MHC-II and T cell costimulatory molecules CD40, CD80, and CD86, all of which are hallmark indicators of DC maturation, but also the expression levels of these markers on a per cell basis as indicated by mean fluorescence intensity.

Wolfberry upregulates IL-12 and TNF-α production by DCs. Activated DCs produce proinflammatory cytokines, which play an important role in regulating the immune response. The ability of DCs to produce these cytokines is often used as an indicator to assess DC activity. In vitro, wolfberry extract–treated DCs induced more proinflammatory cytokines IL-12 and TNF-α compared with control (**Fig. 3***A*, *B*). These results indicate that wolfberry increases functional maturation of DCs,

which is consistent with the increased phenotypic maturation of DCs (Table 1) after wolfberry treatment.

Wolfberry downregulates DC endocytosis. DCs have strong endocytic capability, which is necessary for efficient antigen uptake, but they lose this function when they become mature. Thus, the endocytosis assay is commonly used as a marker to assess DC maturation. Consistent with wolfberry-induced changes in other DC maturation and activation markers, the endocytic capability of DCs was significantly reduced when they were treated with wolfberry extract (**Fig. 4**). These results further confirm wolfberry's ability to promote DC maturation and activation.

Wolfberry-treated DCs enhance antigen-specific T cell response in vivo. The main function of DCs as the most important antigen-presenting cell is to process and then to present specific antigens to T cells to induce their activation and effector function. After finding that wolfberry promoted DC maturation and activation in aged mice, we further investigated whether this would result in a corresponding change in their antigen-presenting function. We conducted adoptive transfer experiments and found that 7 d after wolfberry-treated, $OVA_{323-339}$-pulsed DCs were transferred to the recipient mice, T cell proliferation (**Fig. 5***A*) and CD4$^+$ T cell production of IFN-γ (Fig. 5*B*) and IL-4 (Fig. 5*C*) in response to $OVA_{323-339}$ restimulation were significantly increased compared with the mice that received the control DCs (without wolfberry). These results suggest that wolfberry may enhance DCs' antigen-presenting function, leading to a higher level of antigen-specific T cell effector function involving at least T helper (Th) 1 and Th2 responses.

Discussion

In this study, we report that dietary supplementation with wolfberry could enhance the protective effect of influenza vaccine when the host encounters a subsequent infection. This effect of wolfberry is related to its ability to increase antibody production as well as to promote DC activation and their antigen-presenting capability, which results in a stronger antigen-specific T cell response. These findings provide addi-
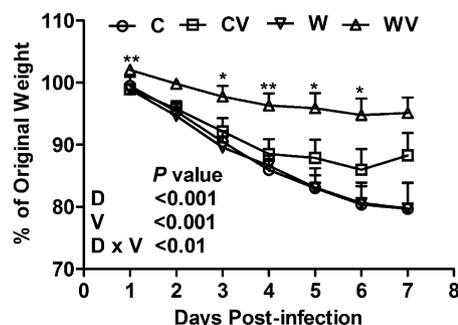

FIGURE 2 Dietary wolfberry (W) enhanced the vaccine's preventive effect against weight loss due to influenza infection in C57BL/6 mice. Values are means ± SEMs, $n = 6–11$/group at each time point. Statistical results shown in the figure are generated from repeated-measures ANOVA. Significantly different from the control (C) group on the same postinfection day by t test: *$P < 0.05$ and **$P < 0.01$. CV, control + vaccine; D, diet; D × V, diet and vaccine interaction; V, vaccine; WV, wolfberry + vaccine.

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TABLE 1 Effect of wolfberry on expression of DC maturation markers in C57BL/6 mice[1]

| Maturation markers | Wolfberry | | | | | LPS[2] |
	0 mg/L	100 mg/L	200 mg/L	400 mg/L	800 mg/L	
CD11c^{+}CD40^{+}						
%	38.9 ± 1.5[a]	47.8 ± 1.6[b]	51.9 ± 1.5[c]	54.9 ± 0.8[c]	60.3 ± 1.0[d]	88.9 ± 0.4
MFI, ×10[2]	167 ± 13.3[a]	209 ± 19.0[a,b]	211 ± 14.8[a,b]	253 ± 18.6[a,b]	290 ± 17.8[b]	571 ± 33.2
CD11c^{+}CD80^{+}						
%	34.9 ± 0.9[a]	42.6 ± 0.5[b]	49.8 ± 0.3[c]	56.0 ± 0.6[d]	64.5 ± 0.7[e]	80.3 ± 0.3
MFI, ×10[2]	83.3 ± 0.6[a]	121.7 ± 1.5[b]	161.7 ± 5.6[c]	172.0 ± 4.7[c]	190.3 ± 6.2[d]	191.7 ± 5.2
CD11c^{+}CD86^{+}						
%	38.4 ± 0.8[a]	41.4 ± 1.1[b]	49.3 ± 0.8[c]	53.0 ± 0.8[d]	64.0 ± 1.0[e]	70.1 ± 1.0
MFI, ×10[2]	32.0 ± 4.3[a]	42.8 ± 6.0[a]	56.2 ± 1.0[b]	66.4 ± 2.8[b]	80.4 ± 0.8[c]	115.1 ± 5.3
CD11c^{+} MHC-II^{+}						
%	59.4 ± 1.1[a]	60.3 ± 0.8[a]	64.3 ± 0.5[b]	65.2 ± 0.7[b]	67.0 ± 0.7[b]	79.7 ± 0.8
MFI, ×10[3]	21.8 ± 7.2[a]	40.7 ± 7.7[a]	65.1 ± 14.3[a]	194.0 ± 19.9[b]	504.3 ± 39.1[c]	559.0 ± 39.0

[1] Values are mean ± SEM percentages of DCs expressing indicated markers and MFI, $n = 3$. Means in a row without a common letter differ, $P < 0.05$. CD, cluster of differentiation; DC, dendritic cell; MFI, mean fluorescence intensity; MHC-II, major histocompatibility complex class II.

[2] LPS (100 μg/L) was used as the positive control.

tional evidence supporting the suggested immunoenhancing effect of consuming wolfberry. Our results have also further extended previous studies by demonstrating, for the first time to our knowledge, that dietary wolfberry–induced enhancement of immune responses, including antibody production, can be translated into better protection against a subsequent infection.

Immunosenescence, one of the most commonly observed, age-associated changes in the body, involves both innate and adaptive immune functions (9,28–30). The age-associated alterations in the immune system are believed to be responsible for a progressive deterioration in its ability to respond to infection and to develop an adaptive immunity after vaccination, both of which are associated with a higher mortality in the elderly (31–33). Thus, developing strategies to reverse these age-related changes would have a great impact on public health, particularly in the elderly. As a promising nutritional approach, a recent study reported that elderly persons who consumed wolfberry for 3 mo (13.7 g/d in the form of the same milk-based preparation of wolfberry used in the current study) had higher serum influenza-specific antibody concentrations after receiving an influenza vaccine compared with age-matched elderly individuals in the placebo group (25). To further support these findings, in the current study we used an influenza infection animal model and determined the impact of wolfberry on antibody production in aged mice as well as wolfberry's ability to enhance the protective effect of flu vaccine. Consistent with the above-mentioned human study, wolfberry increased antibody titers in aged mice after they received influenza vaccine. This increase in the concentration of influenza-specific antibodies was accompanied by a significantly smaller weight loss, an objective indicator of infection severity, in the vaccinated mice fed wolfberry compared with those that received vaccination alone. These results suggest that the wolfberry-induced enhancement of influenza vaccine efficacy could result in reduced disease severity upon subsequent exposure to the virus. The wolfberry supplement used in the current dietary intervention study contained 50% wolfberry, and thus this form of wolfberry added at 5% to the diet is equivalent to 2.5% dried wolfberry in the diet. As we previously described in more detail (23), using the isocaloric calculation, this dose is equivalent to 0.18 g/kg body weight in humans, or 12.6 g/d consumed by a

person weighing 70 kg. This dose is easily achievable and is within the dose range of the same form of wolfberry that was used in a previous study in which higher antibody titers in response to flu vaccine compared with the control group were reported (25). Thus, our results have translational value for humans.

Vaccination is a process in which several components of the immune system are involved. The effect of wolfberry on antibody production after vaccination could be mediated by its effect on various immune cells. A previous study found that dietary wolfberry supplementation enhanced both in vivo (delayed-type hypersensitivity) and ex vivo (T cell proliferation) T cell response to specific antigens, but it did not affect mitogen-induced T cell or B cell proliferation (24). Several studies have shown that in vitro supplementation with polysaccharides or polysaccharide-protein complex isolated from wolfberry induces phenotypic and functional maturation of DCs, and wolfberry-treated DCs have stronger immunogenicity (21,22,34). Together, these results suggest that wolfberry's enhancing effect on influenza vaccination may be mainly mediated through upregulated DC maturation and function during the development of adaptive immunity against influenza virus.
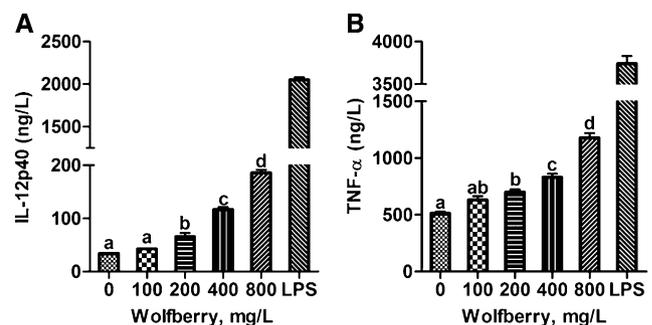

FIGURE 3 Wolfberry upregulated interleukin (IL)-12 and tumor necrosis factor-α (TNF-α) production by dendritic cells from C57BL/6 mice. Values are means ± SEMs, $n = 3$. Means without a common letter differ by 1-factor ANOVA, $P < 0.05$. LPS served as the positive control.

To determine if wolfberry could improve the antigen-presenting function of DCs in aged mice, we first used an in vitro cell-based model to test how the presence of wolfberry might affect DC development from their BM precursors. Consistent with the previous studies mentioned above, we found that wolfberry dose-dependently increased DC maturation on the basis of the changes in both phenotype (MHC-II and costimulation markers) and functionality (cytokine production and phagocytosis). To validate whether wolfberry-treated DCs could in fact perform better at processing and presenting a specific antigen to T cells, thereby leading to a stronger T cell response to this antigen, we conducted an adoptive transfer experiment. In this experiment, DCs were pulsed with the antigen OVA peptide in the presence or absence of wolfberry. These DCs were then injected into the recipient animals to induce the generation of a specific colony of T cells whose receptors would recognize and respond to OVA. To assess wolfberry's effect on the antigen-presenting activity of DCs, we chose 2 specific effector functions of these antigen-specific T cells: clonal expansion and cytokine production. Upon being restimulated in vitro with OVA, T cells isolated from the mice receiving wolfberry-treated, OVA-pulsed DCs had a higher proliferative response compared with the T cells from mice receiving the OVA-pulsed DCs without wolfberry treatment. Likewise, wolfberry-treated DCs induced CD4$^+$ T cells to produce more IFN-γ and IL-4. Given that IFN-γ and IL-4 are hallmark cytokines for Th1 and Th2 cells, respectively, these results suggest that both Th1 and Th2 responses may have contributed to the wolfberry-induced enhancement of antibody production and reduced disease severity after influenza vaccination.

It is unclear what bioactive constituents present in wolfberry contributed to its immunomodulating effects as observed in this and other previous studies. Wolfberry is rich in polysaccharides, carotenoids (zeaxanthin and β-carotene), and vitamins (vitamin C, riboflavin, and thiamin); and it also contains a variety of flavonoids, certain FAs, and amino acids (35). Among these components, polysaccharides are not only quantitatively predominant but are also proposed to be the most important group of molecules responsible for wolfberry's immunomodulatory properties. Although wolfberry contains high concentrations of the antioxidants zeaxanthin and vitamin C, there has been no solid evidence suggesting that these compounds significantly affect adaptive immune response. The other minor components
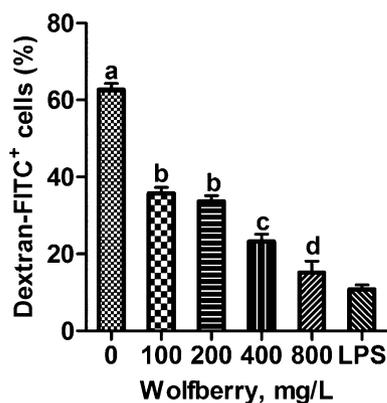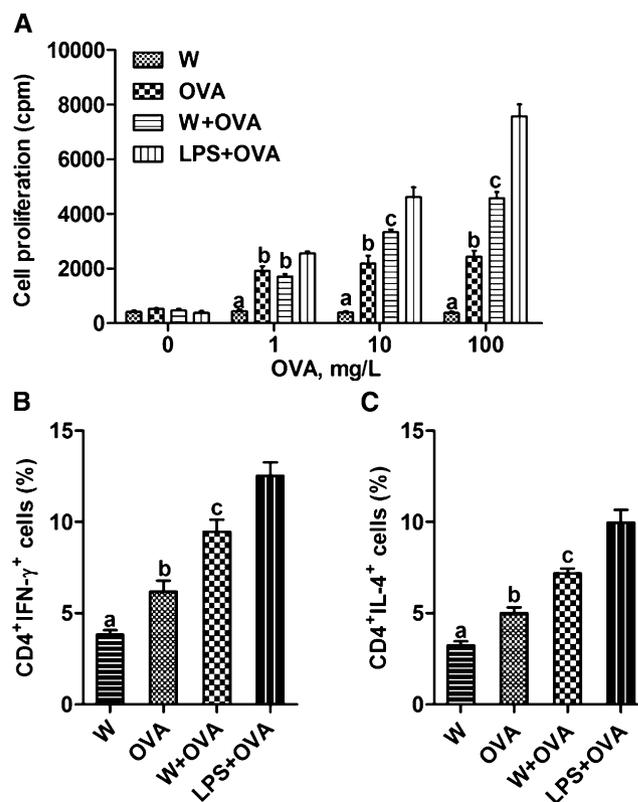

FIGURE 5 Wolfberry (W)-treated dendritic cells (DCs) from C57BL/6 mice enhanced antigen-specific T cell response in vivo. Splenocytes from the DC adoptive transfer recipient mice were restimulated in vitro with ovalbumin$_{323-339}$ peptide to determine antigen-specific T cell proliferation (A) and production of cytokine interferon-γ (IFN-γ) (B) and interleukin (IL)-4 (C) by cluster determinant 4$^+$ (CD4$^+$) T cells. Values are means ± SEMs, n = 5. Means without a common letter differ by 1-factor ANOVA, P < 0.05. LPS+OVA served as the positive control. cpm, counts per minute; OVA, OVA$_{323-339}$ peptide.

mentioned above are presently at such low concentrations that they are unlikely to have any significant effect. Thus, we speculate that wolfberry's effects observed in the current study may be largely attributed to its polysaccharides. The polysaccharides in wolfberry are mainly present as β-glycans in structure, with a backbone of 1–6 β-galactosyl residues, one-half of which are substituted at C-3 by galactosyl or arabinosyl groups (36). However, it is currently unknown how these proposed bioactive compounds act on immune cells to change their function in response to influenza infection, and thus future studies are needed.

In summary, in the current study, we demonstrated that dietary wolfberry may increase the efficacy of influenza vaccine as evidenced not only by the elevated serum antibody concentrations but also by the reduced severity of the disease after infection. This effect of wolfberry may be mediated through promoting DC maturation and antigen-presenting function, resulting in stronger adaptive immunity directed at the influenza virus. These results suggest that dietary supplementation with wolfberry may potentially be used as a complementary approach to improve compromised adaptive immunity and influenza vaccine efficacy in the elderly.

FIGURE 4 Wolfberry downregulated endocytic capability of dendritic cells from C57BL/6 mice. Values are means ± SEMs, n = 3. Means without a common letter differ by 1-factor ANOVA, P < 0.05. LPS served as the positive control. FITC, fluorescein isothiocyanate.

wrote the manuscript; X.D., J.W., X.N., D.S., D.W., and S.N.M. conducted the research and/or analyzed and helped interpret the data; and S.N.M. had primary responsibility for final content. All authors read and approved the final manuscript.

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