THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 15, Number 4, 2009, pp. 423–430

DOI: 10.1089/acm.2008.0215

In Vivo Effects of Ashwagandha (*Withania somnifera*) Extract on the Activation of Lymphocytes

Jeremy Mikolai, Andrew Erlandsen, Andrew Murison, Kimberly A. Brown, M.S.O.M., William L. Gregory, Ph.D., Padma Raman-Caplan, N.D., and Heather L. Zwickey, Ph.D.

Abstract

Objective: This study investigated the immunologic effects of Ashwagandha (*Withania somnifera*) on four types of immune cells in a human sample to determine the immunologic mechanism.
Design: Five (5) participants consumed 6 mL of an Ashwagandha root extract twice daily for 96 hours. Ashwagandha was administered with *anupana* (whole milk). Peripheral blood samples were collected at 0, 24, and 96 hours and compared for differences in cell surface expression of CD4, CD8, CD19, CD56, and CD69 receptors by flow cytometry.
Results: Significant increases were observed in the expression of CD4 on CD3+ T cells after 96 hours. CD56+ NK cells were also activated after 96 hours as evidenced by expression of the CD69 receptor. At 96 hours of use, mean values of receptor expression for all measured receptor types were increased over baseline, indicating that a major change in immune cell activation occurred across the sample.
Conclusions: Effects on immune cell activation with use of Ashwagandha warrant further study.

Introduction

Ashwagandha (*Withania somnifera* Dunal), has been used for centuries in Ayurvedic medicine to treat diverse ranges of illness. Ashwagandha is a member of the Ayurvedic class of herbs called *rasyana*, long prized for "rejuvenating" effects on health.[1] Ashwagandha is known in the West as an adaptogen, believed to assist the body in achieving appropriate responses to stressors, both acute and chronic.[2,3] Adverse effects associated with chronic stress include decreased insulin sensitivity, irregularities in endogenous glucocorticoids, cognitive impairment, and immunosuppression.[4] Over recent decades, several adaptogens have been investigated for effects on these states of physiologic impairment. Only a handful of *in vivo* human investigations of Ashwagandha have been conducted, and only one was concerned with the effects of Ashwagandha on lymphocyte activity.

Eight (8) clinical studies have examined physiologic effects of Ashwagandha alone or in combination with other interventions. One study using powdered Ashwagandha and milk in children 8–12 years old demonstrated significant increases in RBC hemoglobin and in hand grip strength in children who received Ashwagandha and milk for 60 days as compared to placebo control.[5] Another study in adult men (aged 50–59), displayed significant increases in red blood cell count and hemoglobin count, as well as a significant decrease in erythrocyte sedimentation rate, significant improvements in seated stature, hair melanin, and self-reported sexual function after taking Ashwagandha for 1 year.[6]

The efficacy of an herbomineral formulation containing Ashwagandha was evaluated in participants with osteoarthritis in a double-blind, placebo controlled, crossover study. At the end of the 3-month trial, results demonstrated that the formulation produced significant improvement in both severity of pain and disability scores.[7] Another study using an aqueous extract of Ashwagandha root powder demonstrated a short-term chondroprotective effect on damaged osteoarthritic cartilage matrix in 50% of patients and an *in vitro* protective effect due to inhibition of gelatinase activity of collagenase type 2 enzyme.[8]

Two (2) studies of geriatric populations compared the use of yoga, Ayurveda (including Ashwagandha), and placebo interventions for depression and insomnia. Each of the two studies lasted 24 weeks; neither one demonstrated significant results due to the use of Ayurveda and Ashwagandha.[9,10]

In another small clinical investigation, 6 volunteers with type 2 diabetes mellitus and 6 volunteers with hypercholesterolemia participated in a 30-day intervention using Ashwagandha powder. Significant decreases were demonstrated

Helfgott Research Institute, National College of Natural Medicine, Portland, OR.

in urine volume, urine sodium, serum cholesterol, triglycerides, low-density lipoprotein and very-low-density lipoprotein; a decrease in blood glucose comparable to that of oral hypoglycemic agents was also observed.[11]

In the only study that reported immunologic outcomes, a polyherbal formulation containing Ashwagandha was administered to 36 HIV+ participants for 18 months. Over the trial, participants displayed a significant increase in CD4+ T-cell count and a significant decrease in mean viral load. [12]

Several animal studies have demonstrated the effects of Ashwagandha on the immune response in mice.[13–22] It has been observed to promote significant increase in the proliferation of CD4+ and CD8+ T cells, as well as NK cells.[14,18] Ashwagandha has been shown to induce production of cytokines associated with a specific type of immune response (Th1) known to be beneficial for fighting bacteria and viruses.[13,14,18,19]

Extract of Ashwagandha has been demonstrated to protect mice from lethal doses of *Listeria monocytogenes* and induce lymphocyte proliferation and interferon (IFN)-γ production.[21] Antibacterial activity of Ashwagandha extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* has been documented *in vitro*, as have antifungal properties. Also, four studies demonstrating Ashwagandha's effects against *Plasmodium* spp *in vitro* and in animal models have been documented. These observations add to the evidence regarding potential antimicrobial effects of Ashwagandha.[23–29]

While animal studies and *in vitro* studies suggest that Ashwagandha has beneficial immune effects, translation to humans is complicated because the number of *in vivo* clinical studies is so small and because modern research has left traditional modes of administration largely unexplored; Ashwagandha is traditionally administered simultaneously with *anupana*.

According to Ayurveda, *anupana* is an "after-drink" substance that invigorates, gives contentment, helps proper movement within the body, and helps to promote proper digestion of the substance with which it is given.[30] *Anupana* is believed to help direct the action of the herb to specific body systems and possibly make the herb more bioavailable. In traditional Ayurveda, Ashwagandha is administered with *anupana*; thus, studies that examine the mechanistic effects of Ashwagandha must include this step.

The importance of early phase research in complementary and alternative medicine (CAM) cannot be overstated. Early phase pilot studies and short-term investigations with small sample sizes allow CAM researchers to ascertain the value of pursuing particular investigations without committing undue resources to them.[31] This study was implemented to ascertain the value of a commitment to conducting ongoing clinical research into the mechanisms of Ashwagandha effects on immune function.

The study presented herein examined the effects of Ashwagandha, taken with ultrapasteurized organic cow's milk, on activation and population changes of leukocytes in healthy human participants over a 96-hour period. The purpose of the study was to determine the mechanism of how Ashwagandha might elicit its immune effects. Activation was determined by cell surface receptor expression of the activation marker CD69, concurrent with CD4 and CD3 on CD4 T-helper cells, CD8 and CD3 on cytotoxic T lymphocytes (CTLs), CD19 on B cells, and CD56 on natural killer (NK) cells. Activation of T- lymphocytes, NK cells, and B cells induces CD69 expression on the cell surface.[32–35] Understanding the affects of Ashwagandha on the immune system will allow clinicians to determine the most appropriate times for administration.

Methods

Study design

A nonexperimental design was employed to explore the actions of Ashwagandha on immune function with a baseline assessment, treatment, and follow-up assessments. As an early phase pilot, the design of the study was focused on assessing the feasibility and value of a sustained research investment on this topic. Therefore, it was designed to be conducted in a small sample, without controls or blinding procedures, to test for differences over a short duration. A summary of the study outline is presented in Figure 1.

Participants

The study protocol was approved by the Institutional Review Board (IRB) of the National College of Natural Medicine (NCNM), Portland, OR, IRB approval number: 02202007A. The study was conducted May 2007 through August 2007 at the research laboratory of the Helfgott Research Institute Portland, OR. Five (5) participants took Ashwagandha liquid extract for a period of 96 hours. Recruitment occurred by word of mouth and via paper and electronic notices posted to community message boards accessible to the student body at NCNM. Eight (8) potential participants were interviewed on a rolling basis and were selected according to the inclusion and exclusion criteria below (Table 1). Two (2) male and 3 female participants (age range 23–29 years, mean age 26 years), provided written consent, completed the study, and were compensated for their time.

Botanical sources

Botanical material was procured from Pacific Botanical Certified Organic Herb Farm, USA. The herb was collected by Dori J. Maron, QC manager from Pacific Botanicals Fields, Grants Pass, OR. The botanical specimen was identified as Ashwagandha (*Withania somnifera,* Dunal), by AHP, Certificate Nr. 24. A voucher specimen of the botanical material used to create the study extract is cataloged and preserved at Gaia Herbs, Gaia #115121107.

Extract

Withania somnifera extract was obtained from Gaia Herbs, Brevard, NC. The product liquid was a grain ethanol and spring water extraction of dried, macerated *W. somnifera*, Dunal roots at a ratio of 3:1. The extract was processed by low heat evaporation to a final alcohol concentration of 53.13%. No marker compound was chosen for the extract and no standardizations were made. Certificate of analysis on the product was prepared by Gaia Herbs on October 13, 2006. Microbial testing reported that *Escherichia coli* and *Salmonella* were absent in 10 g; fewer than 10 yeast and fewer

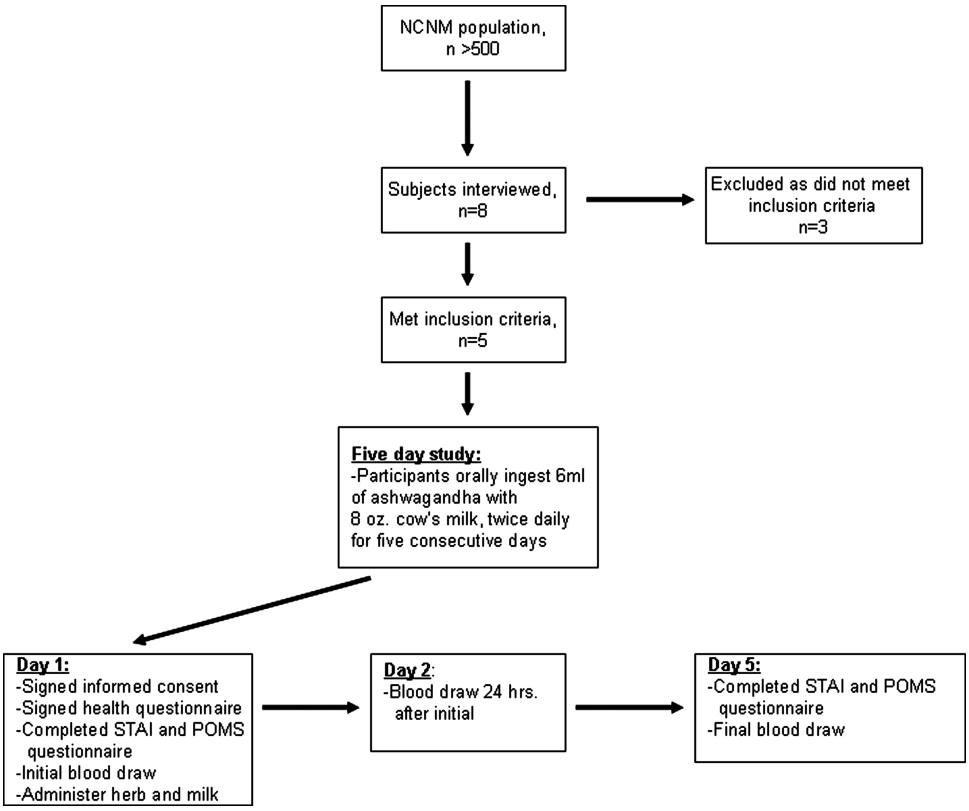

FIG. 1. Study design. POMS, Profile of Mood States; STAI, State-Trait Anxiety Inventory; NCNM, National College of Natural Medicine.

than 10 mold spores were present in total. The bacterial endotoxin/lipopolysaccharide (LPS) concentration of the product was assessed on September 8, 2008 by Limulus amoebocyte lysate test and was found to be 21.0 EU/mL, or approximately 2.1 ng/mL. Participants were supplied with 70 mL of the extract and were instructed to self-administer 6 mL orally twice daily (AM/PM) for 5 days.

Milk

Cow's milk was chosen as the *anupana* in this study because it is readily available and is generally considered, in Ayurveda, the most suitable *anupana* for medicinal substances such as Ashwagandha. Participants were excluded if they self-reported sensitivity to dairy foods during screening. One (1) gallon of ultrapasteurized organic whole milk was provided to each participant on Day 1 of the study. The participants were instructed to drink 8 fluid ounces with each administration of Ashwagandha extract.

Sample collection

Participants provided blood samples at times, $t = 0$, 24, and 96 hours. At each of the three measurements, certified phlebotomists collected blood samples into cell separation tubes containing sodium heparin and a polyester gel plug. Samples were separated by centrifugation at 25°C, 1800 relative centrifugal force (RCF) for 15 minutes. The Buffy coat layer was transferred to a 15-mL conical tube and centrifuged at 4°C, 1500 RPM for 20 minutes, then prepared for analysis by FACScan flow cytometry.

Cell preparation

Leukocytes separated from the centrifuged blood sample were fluorescently labeled using monoclonal immunoglobulin G mouse anti-human antibody specific for each receptor type or isotype control. Samples were analyzed using FACScan flow cytometry to identify and determine the

Table 1. Inclusion/Exclusion Criteria

Inclusion criteria:

- 18–65 years old
- A student at the National College of Natural Medicine

Exclusion criteria:

- Fear or aversion to needles or blood draws
- Recent infection or immunocompromised
- Allergy to cow's milk
- Pregnancy
- Known herb allergies
- Allergies or medical contraindications to alcohol
- Medication contraindications, such as: benzodiazepines, CNS depressants, immunosuppressants, and thyroid hormone and supplements

CNS, central nervous system.

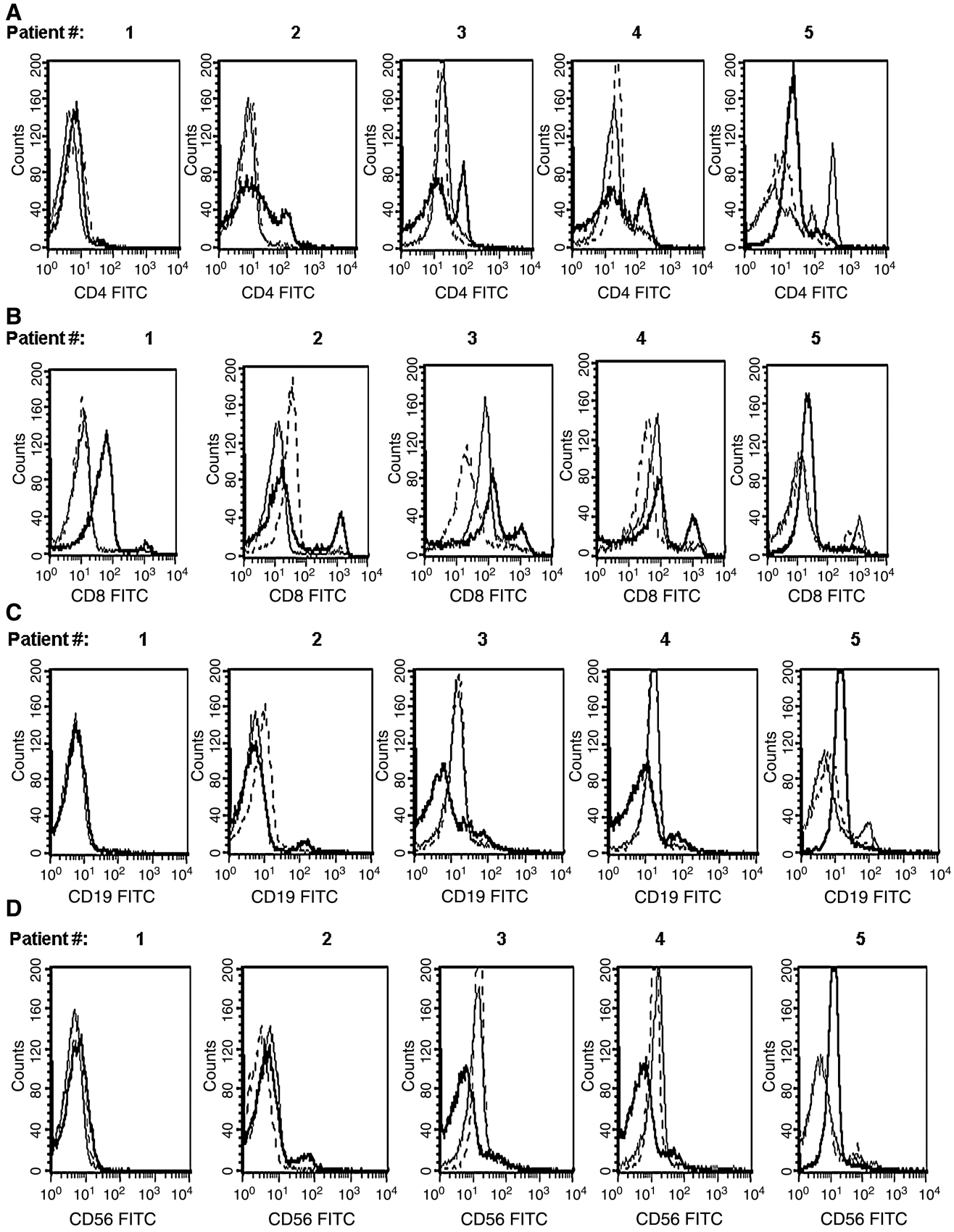

FIG. 2. Count and surface receptor expression at 0, 24, and 96 hours in 5 participants taking Ashwagandha extract and milk. CD4 expression is shown in (**A**), CD8 expression in (**B**), CD19 expression in (**C**), and CD56 in (**D**). Cells were stained with fluorescent antibody as described in the Methods section. Dashed line represents t = 0 hours, thin line represents t = 24 hours, and bold line represents t = 96 hours. FITC, fluorescein isothiocyanate conjugated.

number of various immune cells present per 20,000 cell sample, as well as their relative expression of certain surface receptors including CD69. This procedure was repeated at 0, 24, and 96 hours immediately following the blood sample collections from each participant.

Primary analysis

To control for alphawise error, a multivariate analysis of variance (MANOVA) was performed on the mean fluorescent intensity (MFI) data from the four antibody-labeled cell types. Initially, a MANOVA was used to compare receptor expression at baseline to receptor expression at 24 hours. If that result was statistically significant, then a comparison of receptor expression at 24 hours to 96 hours was planned. If the initial comparison was not significant, then a comparison of receptor expression at baseline to receptor expression at 96 hours was planned. Paired *t*-tests were used to determine *p*-values for CD69 expression and individual receptor expression on individual cell types.

Results

Surface receptor protein expression and the change in that expression from the baseline level over time can be used to measure cell activation and changes in their count. Each of the four types of immune cells measured in this study is identified by a unique combination of receptors. For example, CD4+ and CD8+ T cells were identified by the expression of the CD3 receptor (T-cell receptor signaling complex) as well as either CD4 or CD8. Fluorescent antibody bound to each of the receptors is detectable by flow cytometry. An increase in MFI between two time points demonstrates activation. This increase in receptor expression is shown graphically by a shift to the right on the *x*-axis on a histogram (Fig. 2), or in the area of density on a dot plot (Fig. 3). Increases in cell population are measured by an increase in the size of the peak on a histogram or an increase in the number of cells in the gate on a dot plot (Fig. 3). Activation of CD4+ T cells, CD8+ T cells, B cells, and NK cells is further determined by co-expression of CD69 with the cellular markers.

Log transformations were performed on the raw data to reduce variance. The MANOVA comparing receptor expression of CD69 of all four cell types at baseline to receptor expression at 24 hours was not significant, $F(1,4) < 1$, $p > 0.75$, observed power was 0.06. Accordingly, the second contrast was performed comparing baseline to 96 hours. That MANOVA was significant, $F(1,4) = 15.1$, $p < 0.02$, with a moderate to large effect size, $\eta^2 = 0.79$. Observed power was 0.82. Representative data for CD69 expression on CD4 T cells is shown in Figure 3. Means for CD69 expression on CD4 T cells, CD8 T cells, NK cells, and B cells at baseline, 24 hours, and 96 hours are displayed in Table 2.

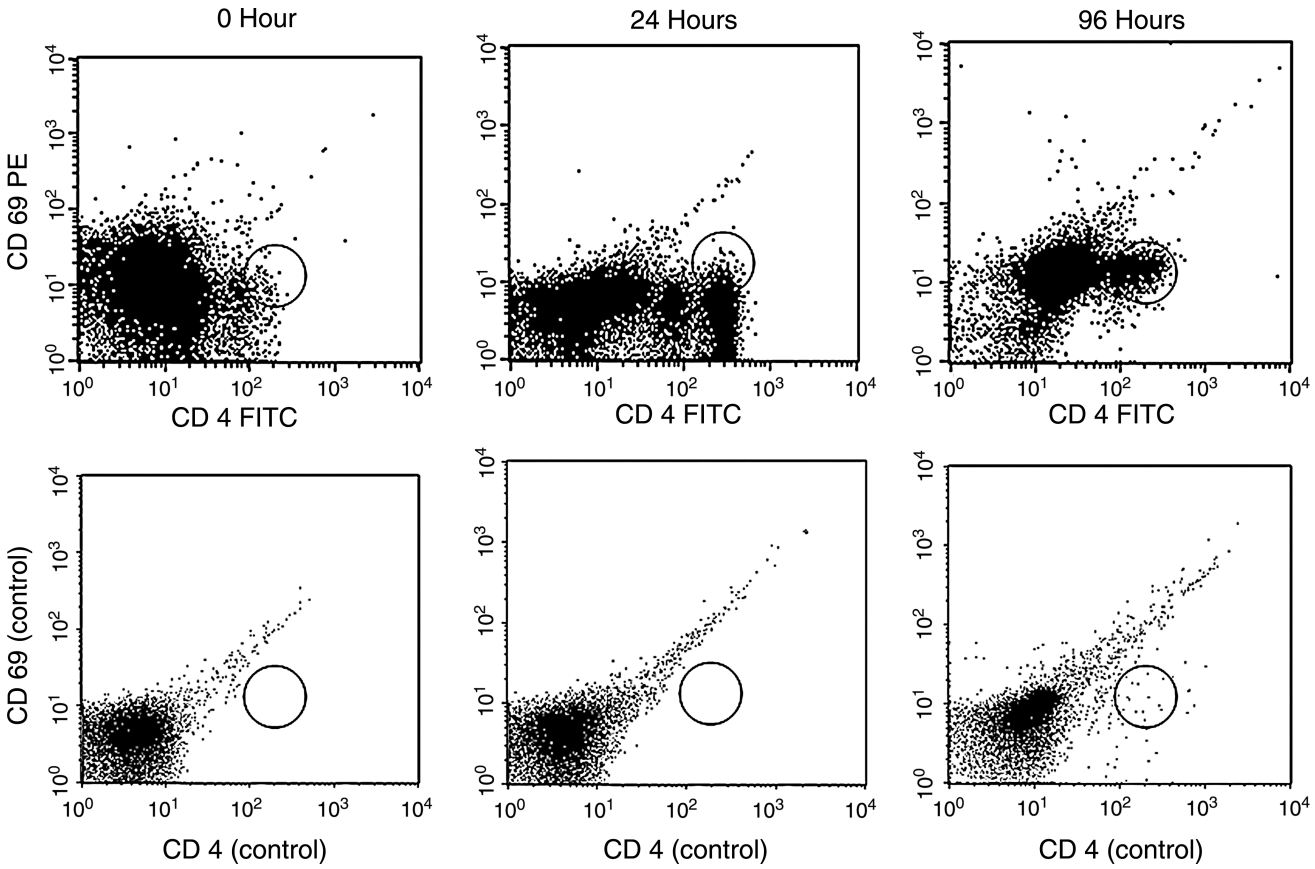

FIG. 3. Dot plot representation of CD4 expression versus CD69 expression data (above) and CD4 isotype control versus CD69 isotype control data (below), in participant 5 taking Ashwagandha over 96 hours. Each dot represents an individual cell stained for CD4 and CD69 (above) or an isotype control cell which received no staining (below).

Table 2. Average Mean Fluorescent Intensity

	CD4		*CD4–69*		*CD8*		*CD8–69*	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
0 hr	786	(846)	52	(22)	1238	(1192)	51	(34)
24 hr	2089	(3162)	32	(10)	1142	(1013)	63	(48)
96 hr	3326	(1858)	267	(271)	4333	(3631)	412	(615)
%	423		513		350		808	
	CD19		*CD19–69*		*CD56*		*CD56–69*	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
0 hr	490	(434)	55	(32)	431	(638)	53	(31)
24 hr	761	(953)	33	(14)	497	(520)	162	(130)
96 hr	702	(380)	287	(292)	701	(717)	169	(32)
%	143		522		163		319	

The final line represents the percent increase from 0 hr to 96 hr.
M, mean; SD, standard deviation.

As can be seen from Table 2, all cells had higher receptor expression over time. This indicates activation of the different cell types. The CD56+ NK cell line displayed a significant increase in CD69 expression at 96 hours relative to baseline ($p < 0.01$). Significant increases in CD69 expression were also observed in the CD4+ cells between the 24-hour and 96-hour measurements ($p < 0.02$).

The MANOVA comparing the expression of the individual cell types at baseline to expression at 24 hours was also not significant, $F(1,4) < 1$, $p > 0.50$, observed power = 0.09. The second contrast comparing baseline to 96 hours trended toward significance, $F(1,4) = 5.6$, $p < 0.08$, partial $\eta^2 = 0.58$, observed power = 0.44. Means for CD4 T cells, CD8 T cells, NK cells, and B cells at baseline, 24 hours, and 96 hours are also displayed in Table 2.

As can be seen in Table 2, a significant increase in the MFI of CD4+ T cells was observed at the 96-hour measurement as compared to baseline ($p < 0.05$). The Ashwagandha-induced activation also varied between individuals. For example, in participant 1, Ashwagandha induced an increase in CD8 expression (Fig. 2B), and much smaller changes in CD4 T cells, B cells, and NK cells. In contrast, CD4 expression increased in participants 2–5 (Fig. 2A). In participants 2, 3, and 4, CD4 levels were highest at 96 hours. In participant 5, CD4 levels were highest at 24 hours. Changes in B cells and NKs were much less pronounced (Fig. 2C, 2D). These cells exhibited more similar profiles of activation among participants 2–5.

Discussion

The data in this study demonstrate a significant increase in the number and activation of CD4+ T cells with administration of Ashwagandha and *anupana* over 96 hours. This immunologic mechanism supports work published previously that demonstrated an increase in CD4+ T cells with the administration of Ashwagandha in animal models.[15,18] The observation of CD4+ T-cell population increase in our study corroborates the finding by Usha et al., who observed increased numbers of CD4+ T cells during an *in vivo* clinical trial of a polyherbal drug containing Ashwagandha in HIV+ participants.[14] Our data suggest that the increase in CD4+ T cells in the Usha study may have been due to Ashwagandha in the polyherbal formulation.

Several studies in animal models have displayed a selective promotion of the Th1-dominant immune response with administration of Ashwagandha.[14,18,19,36] This response, characterized by an increase in the number of CD8+ cytotoxic T lymphocytes (CTLs) and a specific cytokine profile (IFN-γ, interleukin-12 [IL-12], and IL-2), is important for defense against microbes. Bani et al. demonstrated that the immune response elicited by use of Ashwagandha in immunosuppressed and nonimmunosuppressed mice displayed significant increases in the typical Th-1 cytokines IFN-γ and IL-2 accompanied by significant increases CD4+ and CD8+ T-cell counts.[13] Consistent with those findings in animal models, our study displays a marked increase in the number and state of activation of CD4+ T cells and CD8+ CTLs. Although the present investigation lacks the supporting evidence of a typical Th-1 cytokine profile that would more fully support it, the observations herein are consistent with the hypothesis that Ashwagandha may be a selective promoter of Th1 immune response.

The action of Ashwagandha on CD56+ NK cells has not been well investigated. Davis et al. demonstrated that NK cell activity was significantly enhanced in both tumor-bearing mice and those without tumors. Antibody-dependent cellular cytotoxicity activity was also enhanced, suggesting an increased NK response.[14] Nemmani et al. published results that demonstrate an increased NK cell response in mice during intervention with a polyherbal formula containing Ashwagandha.[37] However, no human studies have been performed. The activation of NK cells by Ashwagandha suggests that it would be effective in patients with cancer. The cancer-fighting effects of Ashwagandha constitute an area of prolific research regarding the herb to date.[38–42] The possible implications toward cancer fighting properties of Ashwagandha inherent in its ability to promote the activation and increased population of NK cells has remained unexplored. Our data show an increase in activation state of CD56+ NK cells. The CD69 receptor increased 320% from the baseline level at the 96-hour measurement on NK cells, demonstrating a significant increase in CD56+ cell activation.

To determine whether the quantity of bacterial endotoxin (LPS) present in the investigational product in this study may have had an adjuvant effect on cell activation, the authors completed an *in vitro* titration and analysis of the activation of lymphocytes incubated with LPS. Significant activation of CD4+ and CD8+ T cells occurred at an LPS concentration of 1.0 μg/mL and activation of CD56+ NK cells did not occur. The level of endotoxin present in the investigational product in this study was found to be only 0.002% of that which stimulated activation *in vitro* and was consumed orally, a finding suggesting that it is improbable that endotoxin affected the results.

While Ashwagandha has had many traditional and historical uses, there has been a lack of clear evidence regarding the mechanisms of action in humans. This study suggests that Ashwagandha stimulates a clinically relevant augmentation of the immune response by increasing the activation state and population of certain immune effector cells. The mechanisms reported herein suggest clinical benefits including prophylaxis and treatment of infectious disease, especially against viruses and other intracellular parasites. By selective promotion of a Th1 immune response, Ashwagandha may demonstrate clinical effects on atopy, hypersensitivity responses, autoimmune disease, immune deficiency, and other conditions of inappropriate immune balance. Effects of Ashwagandha against cancer may occur by several mechanisms; activation and increased population of NK cells may be an important component of those antitumor effects. More studies elucidating the mechanisms of action of Ashwagandha in humans and the effects of its use on body systems are indicated. While actions on several systems are being investigated, this study makes it clear that effects on immune function are occurring. Subsequent investigations will need to focus on examination of these effects in larger and more diverse samples in randomized controlled trials.

Acknowledgments

The authors would like to thank Gaia Herbs (Brevard, NC), for providing the herb for this study. The study was funded internally by the Helfgott Research Institute at the National College of Natural Medicine.

Disclosure Statement

No competing financial interests exist.

References

1. Kulkarni SK, Dhir A. *Withania somnifera:* An Indian ginseng. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:1093–1105.
2. Bhattacharya SK, Bhattacharya A, Chakrabarti A. Adaptogenic activity of Siotone, a polyherbal formulation of Ayurvedic rasayanas. Indian J Exp Biol 2000;38:119–128.
3. Monograph. *Withania somnifera.* Altern Med Rev 2004;9:211–214.
4. Bhattacharya SK, Muruganandam AV. Adaptogenic activity of *Withania somnifera:* An experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 2003;75:547–555.
5. Venkataraghavan S, Seshadri C, Sundaresan TP, et al. The comparative effect of milk fortified with *aswagandha, aswagandha* and *punarnava* in children: A double-blind study. J Res Ayur Sid 1980;1:370–385.
6. Kuppurajan K, et al. Effect of *guggulu (Commiphora mukul*–Engl.) on serum lipids in obese, hypercholesterolemic and hyperlipemic cases. J Assoc Physicians India 1978;26:367–373.
7. Kulkarni RR, Patki PS, Jog VP, et al. Treatment of osteoarthritis with a herbomineral formulation: A double-blind, placebo-controlled, cross-over study. J Ethnopharmacol 1991;33:91–95.
8. Sumantran VN, Kulkarni A, Boddul S, et al. Chondroprotective potential of root extracts of *Withania somnifera* in osteoarthritis. J Biosci 2007;32:299–307.
9. Krishnamurthy MN, Telles S. Assessing depression following two ancient Indian interventions: Effects of yoga and Ayurveda on older adults in a residential home. J Gerontol Nurs 2007;33:17–23.
10. Manjunath NK, Telles S. Influence of Yoga and Ayurveda on self-rated sleep in a geriatric population. Indian J Med Res 2005;121:683–690.
11. Andallu B, Radhika B. Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera, Dunal*) root. Indian J Exp Biol 2000;38:607–609.
12. Usha PR, Naidu MU, Raju YS. Evaluation of the antiretroviral activity of a new polyherbal drug (Immu-25) in patients with HIV infection. Drugs R D 2003;4:103–109.
13. Bani S, Gautam M, Sheikh FA, et al. Selective Th1 upregulating activity of *Withania somnifera* aqueous extract in an experimental system using flow cytometry. J Ethnopharmacol 2006;107:107–115.
14. Davis L, Kuttan G. Effect of *Withania somnifera* on cell mediated immune responses in mice. J Exp Clin Cancer Res 2002;21:585–590.
15. Davis L, Kuttan G. Suppressive effect of cyclophosphamide-induced toxicity by *Withania somnifera* extract in mice. J Ethnopharmacol 1998;62:209–214.
16. Gautam M, Diwanay SS, Gairola S, et al. Immune response modulation to DPT vaccine by aqueous extract of *Withania somnifera* in experimental system. Int Immunopharmacol 2004;4:841–849.
17. Ziauddin M, Phansalkar N, Patki P, et al. Studies on the immunomodulatory effects of Ashwagandha. J Ethnopharmacol 1996;50:69–76.
18. Khan B, Ahmad SF, Bani S, et al. Augmentation and proliferation of T lymphocytes and Th-1 cytokines by *Withania somnifera* in stressed mice. Int Immunopharmacol 2006;6:1394–1403.
19. Malik F, Singh J, Khajuria, A, et al. A standardized root extract of *Withania somnifera* and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice. Life Sci 2007;80:1525–1538.
20. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera (ashwagandha):* A review. Altern Med Rev 2000;5:334–346.
21. Teixeira ST, Valadares MC, Goncalves SA, et al. Prophylactic administration of *Withania somnifera* extract increases host resistance in *Listeria monocytogenes* infected mice. Int Immunopharmacol 2006;6:1535–1542.
22. Senthilnathan P, Padmavathi R, Banu SM, et al. Enhancement of antitumor effect of paclitaxel in combination with immunomodulatory *Withania somnifera* on benzo(a)pyrene

induced experimental lung cancer. Chem Biol Interact 2006;159: 180–185.
23. Ali NA, Julich WD, Kusnick C, et al. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol 2001;74:173–179.
24. Girish KS, Machiah KD, Ushanandini S, et al. Antimicrobial properties of a non-toxic glycoprotein (WSG) from *Withania somnifera (Ashwagandha).* J Basic Microbiol 2006;46:365–374.
25. Choudhary MI, Dur-e-Shahwar, Parveen Z, et al. Antifungal steroidal lactones from *Withania* coagulance. Phytochemistry 1995;40:1243–1246.
26. Kazmi SU, Ali SN, Jamal SA, et al. New bioactive compounds of plant origin. Pak J Pharm Sci 1991;4:113–123.
27. Kirira PG, Rukunga GM, Wanyonyi AW, et al. Antiplasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi Districts of Kenya. J Ethnopharmacol 2006;106:403–407.
28. Muregi FW, Ishih A, Miyase T, et al. Antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice. J Ethnopharmacol 2007;111:190–195.
29. Dikasso D, Makonnen E, Debella, et al. Anti-malarial activity of *Withania somnifera L. Dunal* extracts in mice. Ethiop Med J 2006;44:279–285.
30. Vagbhatta KR, Srikantha Murthy [transl.]. Vagbhata's *Astanga hrdayam* Quintessence of the Eight Branches of Ayurveda, text English translation, notes, appendix and indices. Varanasi, India: Krishnadas Academy, 1995.
31. Aickin M. The importance of early phase research. J Altern Complement Med 2007;13:447–450.
32. Hamann J, Fiebig H, Strauss M. Expression cloning of the early activation antigen CD69, a type II integral membrane protein with a C-type lectin domain. J Immunol 1993;150: 4920–4927.
33. Lopez-Cabrera M, Santis AG, Fernandez-Ruiz E, et al. Molecular cloning, expression, and chromosomal localization of the human earliest lymphocyte activation antigen AIM/CD69, a new member of the C-type animal lectin superfamily of signal-transmitting receptors. J Exp Med 1993;178: 537–547.
34. Sancho D, Gomez M, Sanchez-Madrid F. CD69 is an immunoregulatory molecule induced following activation. Trends Immunol 2005;26:136–140.
35. Ziegler SF, Ramsdell F, Alderson MR. The activation antigen CD69. Stem Cells 1994;12:456–465.
36. Spelman K, Burns J, Nichols D, et al. Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. Altern Med Rev 2006;11:128–150.
37. Nemmani KV, Jena GB, Dey CS, et al. Cell proliferation and natural killer cell activity by polyherbal formulation, Immu-21 in mice. Indian J Exp Biol 2002;40: 282–287.
38. Winters M. Ancient medicine, modern use: *Withania somnifera* and its potential role in integrative oncology. Altern Med Rev 2006;11:269–277.
39. Ichikawa H, Takada Y, Shishodia S, et al. Withanolides potentiate apoptosis, inhibit invasion, and abolish osteoclastogenesis through suppression of nuclear factor-kappaB (NF-kappaB) activation and NF-kappaB-regulated gene expression. Mol Cancer Ther 2006;5:1434–1445.
40. Mathur R, Gupta SK, Singh N, et al. Evaluation of the effect of *Withania somnifera* root extracts on cell cycle and angiogenesis. J Ethnopharmacol 2006; 105:336–341.
41. Mohan R, Hammers HJ, Bargagna-Mohan P, et al. *Withaferin A* is a potent inhibitor of angiogenesis. Angiogenesis 2004;7: 115–122.
42. Kaileh M, Vanden Berghe W, Heyerick A, et al. *Withaferin a* strongly elicits IkappaB kinase beta hyperphosphorylation concomitant with potent inhibition of its kinase activity. J Biol Chem 2007;282:4253–4264.

Address reprint requests to:
Heather Zwickey, Ph.D.
Helfgott Research Institute
049 SW Porter Street
Portland, OR 97239

E-mail: hzwickey@ncnm.edu