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Immunomodulating activity of CVT-E002, a proprietary extract from North American ginseng (*Panax quinquefolium*)

Meiqi Wang, Larry J. Guilbert, Lei Ling, Jie Li, Yingqi Wu, Sharon Xu, Peter Pang and Jacqueline J. Shan

Abstract

The activity of CVT-E002, an aqueous extract containing mainly oligosaccharides and polysaccharides from North American ginseng (*Panax quinquefolium*), as an immunobooster on murine spleen cells and peritoneal macrophages, was studied in-vitro. CVT-E002 stimulated the proliferation of normal mouse spleen cells, of which the major responding subpopulation was identified as B lymphocytes. CVT-E002 also activated peritoneal exudate macrophages leading to enhanced interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and nitric oxide (NO) production. In addition, CVT-E002 stimulated in-vivo immunoglobulin G (IgG) production in treated mice. These results identify some of the immunomodulating activities of CVT-E002 and suggest its use clinically for the modulation of immune responses.

Introduction

Panax ginseng C. A. Meyer, commonly called ginseng, has been traditionally used to prevent and treat many kinds of diseases in Asia. Ginseng activity has been characterized by biochemical, immunological and pharmacological methods. Two main medicinal fractions derive from ginseng root processing. One fraction is composed of complex water-soluble polysaccharides and oligosaccharides and the other contains ginsenosides.

Water-soluble ginseng polysaccharides and oligosaccharides have a number of effects on immune and host defence functions. This fraction activates macrophages against *Candida albicans* (Tomoda et al 1994), potentiates anti-complement activity (Tomoda et al 1993), induces interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) production in lymphocytes and peritoneal macrophages (Jie et al 1984; Gao et al 1996), stimulates phagocytosis in polymorphonuclear leucocytes (Hu et al 1995), stimulates natural killer-cell activity (Kim et al 1990) and activates components of cell-mediated immunity (Scaglione et al 1990) including interleukin-2 (IL-2) expression (Ma et al 1995). It has also been shown to reduce bacterial load and lung pathology in animal models of cystic fibrosis (Song et al 1997) and to exert potent gastric cytoprotective and anti-ulcer effects (Sun et al 1991; Kiyohara et al 1994).

Polysaccharides isolated from American ginseng (*Panax quinquefolium*) also have a number of biological effects. North American ginseng polysaccharides enhance lymphocyte transformation, induce IFN-γ and IL-1 production with or

CV Technologies Inc., Edmonton Research Park, 9411-20th Avenue, Edmonton, Alberta, Canada T6N 1E5

Meiqi Wang, Lei Ling, Jie Li, Yingqi Wu, Sharon Xu, Peter Pang, Jacqueline J. Shan

Department of Medical Microbiology and Immunology, University of Alberta, Canada

Larry J. Guilbert

Correspondence: J. J. Shan, CV Technologies Inc., Edmonton Research Park, 9411-20th Avenue, Edmonton, Alberta, Canada T6N 1E5

without suboptimal concanavalin A (Con A), and stimulate spleen-cell production of an IL-3-like cytokine activity (Ma et al 1995; Zhuang et al 1996). Significantly, these polysaccharides have a number of effects on immunosuppressed mice: lymphocyte transformation is improved; thymus and spleen weight is increased; peripheral blood leucopenia is reversed and reticuloendothelial cell phagocytosis is enhanced (Zhu et al 1991). Interestingly, there was no effect of North American ginseng polysaccharides on IL-2 activity in this study.

Although perhaps less impressive than the carbohydrate fraction, the other fraction, which contains ginsenosides, has also been shown to possess immune stimulant properties (Kenarova et al 1990; Fan et al 1995; Kang et al 1995; Liu et al 1995; Kim et al 1998). Ginsenoside extracts have also been shown to potentiate the effectiveness of influenza vaccine in the elderly and to reduce the frequency of the common cold (Scaglione et al 1990).

The range of reported immunomodulating activity for ginseng extracts and the apparently contradictory reports may be due to variation in source (Asian and North American, as well as different sites in Asia and North America), different fractionation methods and different laboratory practices. For example, Li et al (1996) reported that coarse polysaccharide from *Panax quinquefolium* could improve lymphocyte transformation and had no obvious effects on IL-2 activity in immunosuppressed mice. However, Lee et al (1997) reported that an acidic polysaccharide isolated from *Panax ginseng* induced the proliferation of T and B cells and that spleen cells became cytotoxic to a wide range of tumour cells without major histocompatibility-complex restriction and induced the expression of mRNA for IL-2, IFN-γ, IL-1α and GM-CSF (granulocyte/monocyte-colony-stimulating factor). In addition, Kim et al (1990) reported that *Panax ginseng* was primarily associated with NK (natural killer) cell activity, but T and B cell responses were not affected. Further, individual manufacturers have rarely compared ongoing production lots for constancy in physicochemical properties and immunomodulating activity. CVT-E002 is a commercially available aqueous extract of North American ginseng subjected to rigorous quality control of formulation. In this first report on the immunomodulating activity of CVT-E002, using mouse as a model, we focus on its modulation of the natural immunity of mononuclear phagocytes and on its effect on humoral immunity (B cell proliferation and steady-state immunoglobulin (IgG) production). We find that CVT-E002 reproducibly increases B cell proliferation in the spleen, increases circulating IgG levels and stimulates primary macrophage production of immune stimulating cytokines IL-1, IL-6 and TNF-α.

Methods

Extraction of ginseng root

The extraction of ginseng root was accomplished as described in the World Intellectual Property Organization Publication WO 99/30725 (Shan et al 1999) to yield the fraction called CVT-E002 (CV Technologies Inc., Edmonton, Canada). This preparation was dissolved in Hanks Balanced Salt Solution (HBSS, GIBCO BRL, Life Technologies) and filter-sterilized through 0.45-μm filters (Millipore, MA). Concentrations of CVT-E002 are given as mass (μg) per mL before filtering.

Mice

BALB/c (which preferentially mount an antibody-mediated response) and C57BL/6 (which preferentially mount a cell-mediated response) mice (6–8 weeks) obtained from the Health Sciences Laboratory Animal Services Facility (University of Alberta, Edmonton, AB) were used. Mice were kept in a restricted-access room and five mice were housed in each cage. Mice were fed standard pellets with tap water freely available. The mice used in all experiments were reviewed and approved by the Health Sciences Animal Policy and Welfare Committee, the Public Health Service Policy on Human Care and Use of Laboratory Animals (Protocol Number 250/04/01) at the University of Alberta. The University of Alberta is guided by, and complies with, the International Guiding Principles for Biomedical Research Involving Animals, Developed by the Council for International Organizations of Medical Science.

Microbiological analysis

All microbiological assays were carried out by using the Aerobic Colony Count to estimate the numbers of viable microorganisms per gram of product (MFHPB-18). Coliforms were detected by a multiple tube fermentation technique, which estimates the most probable number of total coliforms (MFHPB-19), and *Escherichia coli* was enumerated on Petrifilm E. coli Count plates (MFHPB-34). Enumeration of yeasts and moulds in the samples was performed using a surface spread-plate technique (MFHPB-22). The rapid identification of

salmonella was performed using modified semi-solid Rappaport Vassiliadis (MSRV) method (MFLP-75).

Assay of proliferative response

Spleen cells (2×10^5) were cultured with CVT-E002, 0.5 μg mL^{-1} Con A (Sigma), 10 μg mL^{-1} lipopolysaccharide (LPS; Sigma) in RPMI 1640 medium (pH 7.4, GIBCO) containing 10 % FBS (GIBCO) and a 1 % commercial antibiotic mixture of penicillin–streptomycin (GIBCO) at 37°C in humidified 5 % CO_2 in air for 72 h. The cells were labelled for the last 4 h with 1 μCi of tritiated thymidine ([^{3}H]-TdR, New Life Science Products, Inc., Boston, MA) and harvested with an automated sample harvester (Skatron, Sterling, VA). The amount of radioactivity incorporated into DNA in the cells was measured with a liquid scintillation counter (LS6500 Scintillation Counter, Beckman Coulter, Inc., Fullerton, CA). The results are expressed as the mean counts per minute (counts min^{-1}) with standard error of three experiments.

Fractionation of spleen cells

Lymphocytes were passed through an immunoabsorption column (Biotex Co. Ltd., Edmonton, AB) that removes B cells by adherence to anti-mouse immunoglobulin antibody-coated glass beads. The eluant consisted of an enriched population of T cells. For B cell enrichment, the lymphocytes were first treated with 100 × diluted anti-mouse Thy-1.2 antibody (New England Nuclear) at 37°C for 1 h; 6 × diluted rabbit complement (C′; Cedarlane Labs) was then added and incubated at 4°C for 30 min.

Preparation of macrophages

Peritoneal exudate macrophages (PEM) obtained from the peritoneal cavity of C57BL/6 mice injected with 2 mL of 3 % thioglycollate medium (Sigma) three days previously were cultured at 10^6 mL^{-1} in RPMI 1640 medium containing 10 % FBS, 50 mM mercaptoethanol (ICN Pharmaceuticals, Plainview, NY) and penicillin–streptomycin in 96-well culture dishes (Nalge Nunc International, Roskilde, Denmark) at 37°C in humidified 5 % CO_2 in air for 2 h. The adherent cells were cultured for 48 h with 500, 100 and 10 μg mL^{-1} of CVT-E002, respectively, or 10 μg mL^{-1}of LPS in RPMI 1640 medium containing 10 % FBS as described above. The culture supernatant was harvested, filter-sterilized and assayed for IL-1, IL-6, TNF-α and nitric oxide (NO) as described below.

Assay of interleukin-1 (IL-1)

The IL-1 activity in the culture supernatant was quantified using the CTLL/NOB1 cell line co-cultivation assay (Gearing et al 1987; Rappolee & Werb 1992). CTLL (10^4) and NOB1 (4×10^4) cells were cultured together in a final volume of 200 μL of Iscove's Modified Dulbecco's Medium (IMDM, GIBCO) with 5 % FBS (GIBCO) in 96-well flat-bottom microtitre plates (Nalge Nunc International) containing serially diluted test samples and recombinant mouse IL-1β (PharMingen, San Diego, CA) standards. All standard and sample dilutions were carried out in triplicate. The plates were cultured at 37°C in a fully humidified, 5 % CO_2 in air atmosphere for 20 h, then labelled for 4 h with 1 μCi of tritiated thymidine and harvested with an automated sample harvester (Skatron, Sterling, VA). The amount of radioactivity incorporated into DNA in the cells was measured with a liquid scintillation counter as described above. The results are expressed as mean pg mL^{-1} of IL-1 produced by 1×10^6 macrophages ± standard error of three experiments relative to the standard.

Assay of interleukin-6 (IL-6)

IL-6 activity was quantified by the B9 hybridoma cell line assay (Aarden et al 1987). Briefly, log-phase growing B9 cells were harvested and washed three times with PBS, and 2×10^3 were added to individual wells of a 96-well culture dish in a final volume of 100 μL IMDM made 5 % in FBS. The 100-μL volume contained serially diluted test samples and recombinant mouse IL-6 (PharMingen) as the standard. The plates were cultured for 68 h at 37°C as previously described, then labelled for 4 h with 1 μCi of tritiated thymidine and harvested with an automated sample harvester (Skatron). The amount of radioactivity incorporated into DNA in the cells was measured by liquid scintillation as described above. The results are expressed as ng mL^{-1} of IL-6 ± standard error produced by 1×10^6 macrophages in three experiments relative to the IL-6 standard.

Assay of tumour necrosis factor-α (TNF-α)

TNF activity was quantified by the L929-8 bioassay as previously described (Branch et al 1991). Briefly, L929-8 cells (10^6 mL^{-1}) were added to individual wells of a 96-

well culture dish in IMDM containing 5% FBS and 2 μg mL^{-1} of actinomycin D (Sigma) in a final volume of 50 μL, then incubated at 37°C for 2 h. Test samples and standards (recombinant mouse TNF-α, PharMingen) in a final volume of 50 μL were added to the wells and the plates were incubated at 37°C for 24 h. Neutral Red (Sigma) was added to all wells and the plates were incubated at 37°C for a further 2.5 h. The plates were washed twice with 200 μL of PBS, 100 μL of 50% ethanol in 0.05 M NaH_2PO_4 was added to each well, the plates were agitated for 5 min at room temperature and the absorbance at 570 nm wavelength was measured on a microtitre plate reader (Labsystems Multiskan MCC, Helsinki, Finland). All samples and standards were carried out in triplicate and the results are expressed as mean pg $\pm$ standard error of three experiments.

The Griess reaction for nitrite

NO production was assessed as nitrite production by the Griess reaction as previously described (Smith et al 1999). Briefly, 50 μL of culture fluid from each sample and $NaNO_2$ standard were transferred to wells of a 96-well flat-bottom microtitre plate and serially diluted, and 50 μL Griess reagent (50 μL of 1% sulfanilamide, 50 μL of 0.1% NEDD in 2.5% phosphoric acid) added to all wells. The absorbance at 550 nm wavelength was measured on a microtitre plate reader (Labsystems Multiskan MCC). The results of three experiments are expressed as μM of nitrite produced by 10^6 macrophages (mean $\pm$ standard error) relative to the standard.

In-vivo IgG assessment

CVT-E002 was orally administered to BALB/c mice once daily for 7 days. After 7 days of treatment, blood was collected from tail veins and centrifuged. The serum was retained for the measurement of immunoglobulin G (IgG) by enzyme-linked immunosorbent assay (ELISA). For the ELISA, flat-bottomed microtitre plates (Costar E.I.A./R.I.A. plates; Cambridge, MA) were coated with goat anti-mouse IgG (Sigma, dissolved in 0.02 M Tris buffer) at 37°C for 2 h. After blocking with 1% bovine serum albumin (BSA, Sigma) in PBS, test serum was added and the plate incubated at room temperature for 2 h. The plate was then washed 5 times with PBS containing 0.05% Tween (PBST), and peroxidase-conjugated goat anti-mouse IgG (Sigma; dilution, 2000 $\times$) in 1% BSA–PBST was added. The plate was incubated at room temperature for 1 h and washed five times with PBST; then ABTS solution (ABTS peroxidase substrate with peroxidase solution B (KPL, Gaithersburg, MD) was added. After 15–20 min at room temperature, the absorbance at 405 nm was measured with a microtitre plate reader (Labsystems Multiskan MCC). The results are expressed as mean $\pm$ standard error of five mice compared with a mouse IgG standard (Sigma).

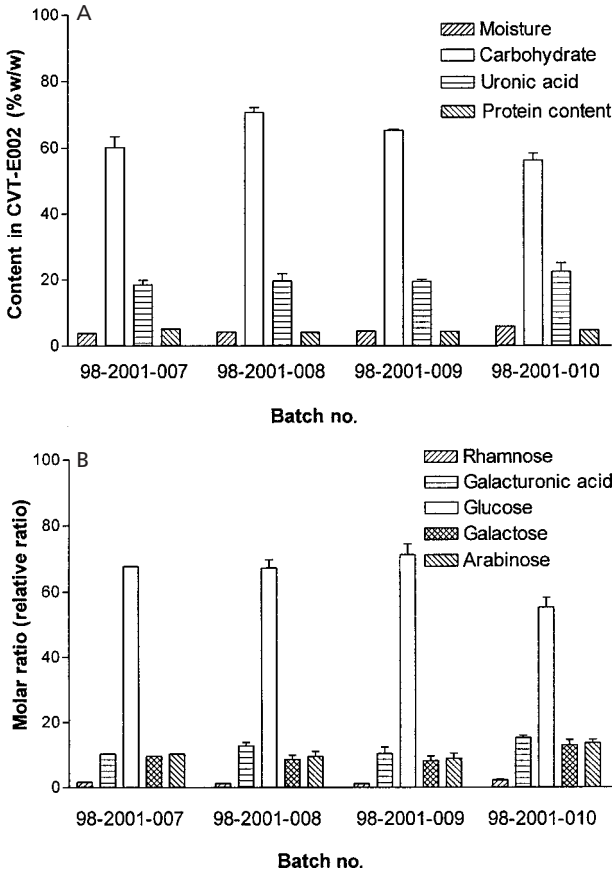

Figure 1 Physicochemical properties and sugar content of CVT-E002. A. Physicochemical properties. Moisture content was determined using method 44-15A of the American Association of Cereal Chemists (once). Protein content was determined using a modification of the Lowry Folin reagent assay (once). Total carbohydrate content was measured by phenol–sulfuric acid method (three times). Uronic acid content was determined by the *m*-hydroxydiphenyl method (three times). The histograms depict the means $\pm$ s.d. of three experiments. B. Component sugars. A sample of CVT-E002 (20 mg) solution in 2 M HCl (2 mL) was heated at 95–100°C for 6 h to liberate its component sugars. The liberated component sugars were derivatized with MPP (3-methyl-1-phenyl-2-pyrazolin-5-one) and analysed by HPLC. Batch 98-2001-007 was analysed once. Another three batches were each analysed three times; the histograms depict the means $\pm$ s.d. of three experiments.

Statistical analysis

Results were expressed as the mean ± the standard errors (s.e.) of three experiments in bioassays and were compared using analysis of variance (ANOVA and Newman-Keuls test). Differences were significant at $P < 0.05$.

Results

Physicochemical analysis of CVT-E002 preparations

The physicochemical properties (moisture, pH, carbohydrate content, uronic acid content and protein content) of four consecutive batches of CVT-E002 prepared in 1998 are shown in Figure 1A. The average variation in these properties was 8.8 ± 5.5%, with the largest variation being in moisture (17.5%), followed by carbohydrate (8.6%), protein (8.4%), uronic acid (7.3%) and pH (2.3%). The carbohydrate content was further broken down into constitutive types (Figure 1B). By the criterion of percent mass, the most abundant carbohydrate was glucose, followed by galacturonic acid, arabinose, galactose and rhamnose. The average variation in carbohydrate subtypes was 20.6%, with the largest variation being with rhamnose (32.7%) followed by galactose (22.1%), arabinose (19.8%), galacturonic acid (19.1%) and glucose (10.7%). The microbiological analyses were also quite consistent, with standard plate counts being low (100–900 per gram) and coliforms, *E. coli*, yeast/mould and salmonella being undetectable in all four batches (Table 1).

The effect of CVT-E002 on mouse spleen cell proliferation

Unfractionated spleen cells from BALB/c mice were cultured with LPS and three different concentrations of CVT-E002 for three days and the relative proliferative response measured by uptake of [^{3}H]-TdR. CVT-E002 stimulated a proliferative response in normal spleen cells in a dose-dependent manner (Figure 2).

To determine which subpopulation was responding to CVT-E002, spleen cells were fractionated using anti-Thy-1 and complement (C′) (to eliminate T lympho-

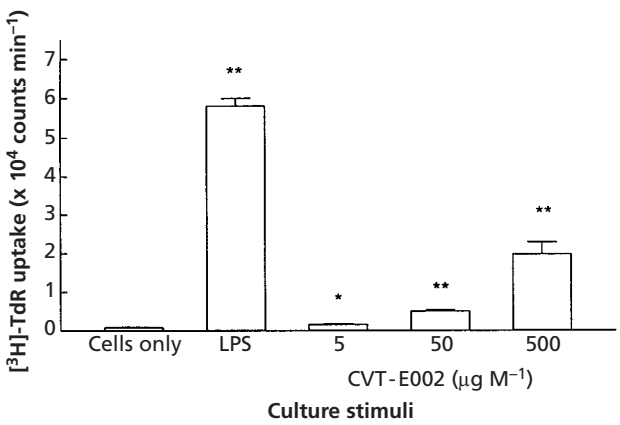

Figure 2 Effect of CVT-E002 on murine spleen cell proliferation. BALB/c spleen cells (2×10^5) were cultured with LPS (10 μg mL^{-1}) or different concentrations of CVT-E002 for three days. The cells were labelled with 1 μCi [^{3}H]-TdR for the last 4 h and harvested, and [^{3}H]-TdR incorporation was measured. The results are expressed as mean counts min^{-1} of [^{3}H]-TdR ± s.e. of three experiments. $^*P < 0.05$, $^{**}P < 0.01$ relative to cells only.

Table 1 Microbiological analysis in batches.

Microbiological analysis[a]	98-2001-007	98-2001-008	98-2001-009	98-2001-010
Standard plate count (g^{-1})[b]	900	500	600	100
Coliforms (g^{-1})[c]	< 10	< 10	70	< 10
E. coli (g^{-1})[d]	negative	negative	negative	negative
Yeast and mould (g^{-1})[e]	< 10	< 10	< 10	< 10
Salmonella[f]	negative	negative	negative	negative

[a]All microbiological assays were carried out by Norwest Soil Research Ltd according to the official Health Canada methods for microbiology and foods, Health Protection Branch. [b]Standard plate count was performed using Aerobic Colony Count to estimate the numbers of viable microorganisms per gram of product. [c]Coliforms were detected by a multiple tube fermentation technique which estimates the most probable number of total coliforms. [d]*E. coli* was enumerated on Petrifilm E. coli Count plates. [e]Enumeration of yeast and mould in products was performed using a surface spread-plate technique. [f]The rapid identification of salmonella was performed by using the modified semi-solid Rappaport Vassiliadis method.

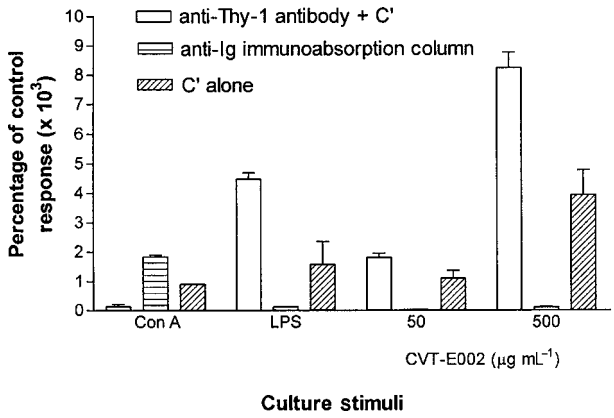

Figure 3 Response of B cells to CVT-E002. Murine spleen cells treated with anti-Thy-1 antibody + C′, an anti-Ig immunoabsorption column or C′ alone were cultured with CVT-E002, Con A (0.5 μg mL^{-1}) or LPS (10 μg mL^{-1}) for three days. The cells were labelled with 1 μCi [^{3}H]-TdR for the last 4 h and harvested. [^{3}H]-TdR incorporated into lymphocytes was measured by a liquid scintillation counter. The results are expressed as mean percentage of control ± s.e. compared with C′ alone treated in three experiments.

cytes) or by passing them through an anti-Ig immunoelimination column (to deplete B lymphocytes); then they were assessed for proliferative responses to LPS (a preferential B cell mitogen), Con A (a preferential T cell mitogen) and CVT-E002 (Figure 3). Unfractionated cells (treated with C′ only) responded well to all three stimuli. Anti-Thy-1 antibody and C′ treatment completely abrogated the Con A response; however, the response to LPS and CVT-E002 remained strong. On the other hand, depletion of B cells with the immunoabsorption column almost completely eliminated the response to both LPS and CVT-E002 although the response to Con-A was enhanced. These data show that the methods for T and B lymphocyte depletion were effective and suggest that the responder cells to CVT-E002 are B cells. Although all CVT-E002 preparations were found to be essentially free of microbial contamination (see above), we nonetheless determined whether the biological responses of spleen cells might be mediated by low levels of LPS in the preparations. We therefore compared the response of cells from BALB/c and C3H/HeJ mice, the latter of which are hyporesponsive to LPS (Rosenstreich et al 1978). We found that BALB/c and C3H/HeJ cells responded comparably to Con A and CVT-E002 but that only BALB/c cells responded to LPS (data not shown).

CVT-E002 stimulated macrophages to produce IL-1, IL-6, TNF-α and NO

Peritoneal exudate macrophages (PEM) were cultured with and without LPS or CVT-E002 and the culture supernatants were assessed for IL-1, IL-6, TNF-α and NO production (Table 2). The highest concentration of CVT-E002 (500 μg mL^{-1}) significantly stimulated PEM production of IL-1 ($P < 0.01$). CVT-E002 at doses of 10 μg mL^{-1} and above significantly increased IL-6 production by macrophages. A dose of 500 μg mL^{-1} of CVT-E002 also significantly increased TNF-α and NO production by PEM ($P < 0.05$, in both cases).

CVT-E002 stimulated IgG production of mice in-vivo

To assess the ability of CVT-E002 to stimulate IgG production in-vivo, groups of five BALB/c mice were treated orally once daily for one week at doses of 18, 6,

Table 2 Effect of CVT-E002 on cytokines production by murine macrophages.

Stimulus[a]	Cytokines released by macrophages (10^6)			
	IL-1[b] (pg)	IL-6[c] (ng)	TNF-α[d] (pg)	NO[e] (μM)
Cells only	253.7 ± 16.9	1.16 ± 0.15	< 0.19	1.93 ± 0.37
LPS	1025 ± 62.1**	15.6 ± 0.20**	20.66 ± 5.63**	39.29 ± 8.74*
CVT-E002 (10 μg mL^{-1})	287.3 ± 22.2	1.90 ± 0.05*	< 0.19	2.21 ± 0.08
CVT-E002 (100 μg mL^{-1})	283.7 ± 28.1	1.99 ± 0.12*	< 0.19	2.86 ± 0.31
CVT-E002 (500 μg mL^{-1})	491 ± 36.4**	3.92 ± 0.25**	2.65 ± 0.64*	6.55 ± 0.05*

[a]Peritoneal exudate macrophages (10^6) of C57BL/6 mice were cultured with or without CVT-E002 (batch 009) at three concentrations for 48 h and culture supernatant was harvested. [b]Assay of IL-1 in the supernatant was performed using CTLL and NOB1 cell lines. [c]Assay of IL-6 was performed using B9 cell line. [d]Assay of TNF-α was performed using L929 cell line. [e]Detection of NO was performed using the Griess reaction. *$P < 0.05$, **$P < 0.01$, compared with cells only.

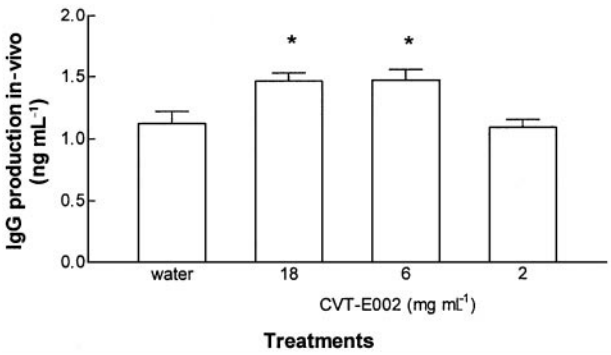

Figure 4 Effect of CVT-E002 on IgG production in-vivo. Three groups (5 mice in each group) of 8-week-old BALB/c mice were fed orally with several concentrations of CVT-E002 once daily for 7 days. A control group (5 mice) was given sterile water and blood collection was performed. IgG production in serum was detected by ELISA. The results were expressed as ng $mL^{-1} \pm$ s.e. $^*P < 0.05$ compared with water only.

or 2 mg per mouse. After one week of treatment, the blood was collected and serum IgG was measured by ELISA. Both the higher concentration treatments of CVT-E002 (18 mg and 6 mg daily) significantly increased IgG production by mice in-vivo ($P < 0.05$, Figure 4).

Discussion

In the past decade, increased attention has been focussed on the pharmacology and clinical utility of botanical extracts and derivatives as methods of standardization have advanced and the safety and efficacy of botanical derivatives have been increasingly demonstrated (Hu 1977; Liu & Xiao 1992; Eliason et al 1997). Although the characterization, compound recipes and biological activity of polysaccharides from *Panax ginseng* C. A. Meyer have been well studied, systematic comparison of physicochemical properties and biological functions of commercially available ginseng extracts are rare. We have therefore investigated the specific immunomodulating activity of a commercial North American ginseng extract (CVT-E002) to determine the nature of the immunomodulation, and whether the immunomodulation is consistent from preparation to preparation (Table 3). We have focussed this first study on immunoglobulin production by B lymphocytes and on natural immune responses by mononuclear phagocytes. CVT-E002 stimulated the proliferation of normal mouse spleen cells, of which the major responding subpopulation were identified as B lymphocytes. CVT-E002 also activated peritoneal exudate macrophages leading to IL-1, IL-6, TNF-α and NO production. In addition, CVT-E002 stimulated in-vivo immunoglobulin G production in treated mice. These results identify some of the immunomodulating actions of CVT-E002. The most important observation of this study is that this biological response is remarkably consistent between CVT-E002 preparations, both qualitatively and quantitatively. This observation is in accord with the concurrent observation that the physicochemical properties of the CVT-E002 preparations are also uniform.

The chemical characterization of CVT-E002 showed that the extract contained mainly polysaccharide. The component sugars of this polysaccharide, in order

Table 3 Comparison of biological activity in batches of CVT-E002.

Stimulus	Lymphocyte proliferation[a] (counts min^{-1})	Cytokines released by macrophages (10^6)				IgG production[f] in-vivo ($\mu g\ mL^{-1}$)
		IL-1[b] (pg)	IL-6[c] (ng)	TNF-α[d] (pg)	NO[e] (μM)	
Cells only	1013 ± 224.9	253.7 ± 16.9	1.16 ± 0.15	< 0.197	1.93 ± 0.37	399.0 ± 20.9
98-2001-008	43724.3 ± 331039***	379.0 ± 53.5	3.69 ± 0.21**	2.55 ± 0.42*	5.26 ± 0.68**	493.4 ± 28.7**
98-2001-009	53557.0 ± 5784.6***	491.0 ± 36.4**	2.26 ± 0.25**	2.24 ± 0.21*	5.00 ± 0.36**	608.6 ± 43.0**
98-2001-010	90532.3 ± 16324.4***	413.5 ± 53.1	3.92 ± 0.25**	2.65 ± 0.64*	6.55 ± 0.05**	544.0 ± 36.2**

[a]BALB/c spleen cells (2×10^5) were cultured with or without CVT-E002 (100 $\mu g\ mL^{-1}$) for three days. The cells were labelled with 1 μCi [^{3}H]-TdR for the last 4 h and harvested. [^{3}H]-TdR incorporated into lymphocytes was counted by a liquid scintillation counter. The results are expressed as mean of counts min^{-1} and standard error of three experiments. Peritoneal exudate macrophages (10^6) of C57B1/6 mice were cultured with or without CVT-E002 (500 $\mu g\ mL^{-1}$) for 48 h and culture supernatant was harvested. [b]Assay of IL-1 in the supernatant was performed using CTLL and NOB1 cell lines. [c]Assay of IL-6 was performed using B9 cell line. [d]Assay of TNF-α was performed using L929 cell line. [e]Detection of NO was performed using the Griess reaction. [f]Three groups (5 mice in each group) of 6–8-week old BALB/c mice were fed with 20 mg once daily per oral administration for 7 days. A control group (5 mice) was given sterile water and blood collection was performed. IgG in serum was detected by ELISA. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with cells only.

of content, are consistently: glucose > galacturonic acid > arabinose > galactose > rhamnose. This is very different from previously reported data on component sugars; mannose, glucose and xylose were found as component sugars of the polysaccharides isolated from American ginseng (Oshima et al 1987). However, Oshima et al found that their ginseng fraction caused a hypoglycaemic effect and its immunological effects were not evaluated. Zhang et al (1996) and Ma et al (1995) found that polysaccharides from American ginseng enhanced lymphocyte transformation, induced IFN-γ and IL-1 production and stimulated spleen cell production of an IL-3-like cytokine activity; however, the structure of the polysaccharide fraction was not studied.

In this study, CVT-E002 significantly stimulated spleen cell proliferation and increased serum IgG levels in treated mice. The spleen cells responding proliferatively to CVT-E002 appear to have been B lymphocytes since the response was lost when B lymphocytes, but not T lymphocytes, were depleted from the spleen cell preparation. Thus, CVT-E002 stimulation of B lymphocyte proliferation is in accord with its stimulation of serum IgG levels by mature B lymphocytes (plasma cells). Further, the observed stimulation of plasma Ig could arise from an increased pool of plasma cells coming either from the observed stimulation of B lymphocyte proliferation or from CVT-E002 stimulation of IL-6 production, which cytokine stimulates the terminal differentiation of B lymphocytes into plasma cells (Hirano et al 2000). Taken together, these observations suggest that CVT-E002 can affect the outcome of an acquired immune response such as antibody production.

In addition to its effect on plasma cell generation, IL-6 is also an inflammatory mediator that stimulates platelet production and hepatocyte acute phase product production (reviewed by Rosenberg & Gallin 1999). The ability of CVT-E002 to strongly stimulate PEM release of IL-6 (by 180%) fits with its stimulation of PEM production of three other inflammatory mediators, TNF-α, IL-1 and NO. All four of these mediators have important functions in immune inflammatory reactions (Rosenberg & Gallin 1999). More specifically, TNF-α has a profound effect on the direct killing of infected or oncologically transformed cells, stimulation of reactive oxygen and nitrogen species (such as superoxide and NO) production, increasing vascular permeability, and increasing T and B cell homing to infected tissue by activating the vascular endothelium (Orlinick & Chao 1998). IL-1 comes predominantly from mononuclear phagocytes and increases blood flow and fever, stimulates production of other mediators and, like TNF-α, also stimulates adhesion-molecule expression by the vascular endothelium. NO is also very pleiotropic, stimulating blood flow and brain functions, as well as mediating cytotoxicity of intra- and extracellular infectious pathogens and tumour cells (Beckman & Koppenol 1996).

The nature of our experimental protocol for inflammatory mediator expression (examining macrophage activation in-vitro) limits our conclusions as to the effects of CVT-E002 on natural immunity, the immediate response to infectious pathogens. However, all of these mediators can have longer-term effects on acquired immunity or more immediate effects (e.g. enhancing IgG production as described above). Further, given the reproducible effects of this defined and well-characterised preparation of ginseng on macrophage and B lymphocyte function (both potent antigen presenting cells (APC) that can initiate acquired immune responses), the next step is to examine possible effects of CVT-E002 on the direction and magnitude of acquired immune responses. Our working hypothesis is that this, or a similar, preparation may act very broadly to modulate both natural and acquired responses and allow the prevention or treatment of diseases associated with depressed immunity.

In conclusion, we observed that CVT-E002, a well-defined polysaccharide fraction with unique chemical composition isolated from American ginseng, increases spleen B lymphocyte proliferation and serum immunoglobulin production. The fraction also caused significant increases in peritoneal exudate macrophage production of the cytokines IL-1, TNF-α and IL-6 and in the production of the antimicrobial product NO. These data suggest that CVT-E002 can modulate acquired immune response and regulate immune inflammatory reactions. Further studies are needed to confirm the ability of CVT-E002 to affect natural immunity, the immediate response to infectious pathogens.

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