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Research Paper

Human dendritic cells promote an antiviral immune response when stimulated by CVT-E002

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Abstract

Objectives There is interest in developing new compounds to enhance the immune response to airway virus infections. CVT-E002 is a patented ginseng extract shown to decrease symptoms of virus infection in clinical trials. We hypothesized that the mechanism for this antiviral effect could be through modulation of dendritic cells leading to enhanced T-cell activation.

Methods Human monocyte-derived dendritic cells (moDC) exposed to CVT-E002 (or not) were co-cultured with autologous T cells, with or without virus (respiratory syncytial virus or parainfluenza virus). Effects of CVT-E002 on cell function were determined through flow cytometry, 5-bromo-2′-deoxyuridine (BrdU) incorporation and ELISA.

Key findings moDC cultured with CVT-E002 or virus induced greater activation of T cells, as measured by CD25 expression and BrdU incorporation, compared with untreated moDC. Responding T cells were CD4+CD45RO+. Co-cultures of CVT-E002 treated moDC with T cells responded with increased release of Th1-type cytokines (interferon-gamma, tumour necrosis factor and interleukin-12). CVT-E002-treated moDC showed increased expression of CD83, CD80 and CD86. Lipopolysaccharide levels were not detected in CVT-E002 and antagonists for Toll-like receptor-4 did not inhibit CVT-E002-induced moDC maturation.

Conclusions CVT-E002 induced moDC maturation, which caused increased memory T-cell activation and Th1-type cytokine response.

Keywords antiviral; dendritic cell; ginseng polysaccharide; T cell; Th1 and autologous co-culture

Introduction

Most chronic airways diseases such as asthma or chronic obstructive pulmonary disease deteriorate as a result of infections by common cold viruses.[1–3] Considering the life threatening nature of these exacerbations and their economical impact, there is interest in the development of new compounds, which could enhance the immune response for prevention of seasonal airway virus infections.[4]

Ginseng has long been considered as beneficial for the immune system, but scientific studies showing specific mechanisms for the effect of ginseng are relatively scarce. General use of ginseng consists of unpurified ginseng extract of unknown components and concentration, and with low reproducibility. CVT-E002 is a proprietary, water-soluble, polyfuranosyl-pyranosy-saccharide-rich extract derived from the root of the North American ginseng *Panax quinquefolium*.[5] CVT-E002 is sold as a natural health product under the brand name COLD-fX for the prevention and treatment of airway virus infections in humans. It is administered via capsules that dissolve in the gut, therefore avoiding carbohydrate degradation caused by enzymatic activity of the saliva. Due to the process used to purify CVT-E002, it is largely devoid of ginsenosides, also known as saponins. A recent study found evidence that CVT-E002 was safe for paediatric use.[6]

Clinical trials have shown that CVT-E002 reduced the relative risk of influenza and respiratory syncytial virus infection by 89%, as well as the incidence and duration of acute respiratory illnesses in an elderly population.[7,8] Another study of adults (18–65 years of age) saw a significant decrease in total symptom score, duration of cold symptoms, and in the recurrence of respiratory infections while ingesting a daily dose of CVT-E002 for four

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months versus placebo.[9] In a mouse model, CVT-E002 appeared to stimulate the immune system by inducing proliferation of splenic B-cells, increasing circulating immunoglobulin G levels, and stimulating macrophage production of interleukin-1 (IL-1), IL-6, tumour necrosis factor (TNF) and nitric oxide (NO), IL-2 and interferon-gamma (IFN-γ).[5,10]

We have experience with an in-vitro human model co-culturing airway viruses with isolated autologous leucocytes.[11] We hypothesized that CVT-E002 could be promoting an antiviral response through modulation of human dendritic cells, which stimulate T cells toward a Th1-like response. For this project, we have used different high-incidence, common airway viruses: respiratory syncytial virus (RSV) and parainfluenza virus (PIV). These viruses were chosen because they are single-stranded RNA (ssRNA) airway viruses that frequently infect humans throughout their lives, and are associated with asthma exacerbations.

The aim of the study was to elucidate possible immunological mechanisms for the antiviral properties of CVT-E002. We measured the effects of CVT-E002 by analysing its effect on human dendritic cell maturation and T-cell activation in an autologous co-culture system with or without airway virus exposure.

Materials and Methods

Reagents

All leucocytes were grown in complete RPMI (RPMI 1640 with HEPES, supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine and non-essential amino acids; Invitrogen, Burlington, ON, Canada). *Rhodobacter sphaeroides* lipopolysachharide (RS-LPS, Toll-like receptor-4 (TLR-4) antagonist) was purchased from INVIVOGEN (San Diego, CA, USA). Anti-CD3 (PerCP), CD4 (Alexa488), CD8 (PE), CD11c (APC), CD25 (APC), CD45RO (PE), CD80 (FITC), CD83 (FITC), CD86 (PE) and HLA-DR (PE) were from BD Biosciences (Mississauga, Ontario, Canada). CVT-E002 was provided by Afexa Life Sciences Inc. (formerly CV Technologies, Inc., Edmonton, Alberta, Canada), as a powder and was resuspended in serum-free RPMI.

Leucocyte isolation

All work proposed in this study was approved by the Health Research Ethics Board, according to the Helsinki protocol, and the Biosafety Office of the University of Alberta. Whole blood was taken from consenting donors. Since RSV and PIV are common airway infection agents, our donors had immunity to this virus in the form of memory T cells. After red blood cell sedimentation, the leucocyte-rich supernatants were subjected to density centrifugation through Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden).

Lymphocyte purification

Mononuclear cells from the Ficoll were placed on six-well plates in RPMI supplemented with 10% FBS (2 h, 37°C, 5% CO_2). Unattached cells, representing a mixed lymphocyte population, were removed and run through a nylon wool column. This lymphocyte enriched population (>90% pure CD3+ by flow cytometry) was counted and stored at −80°C in FBS with 10% dimethyl sulfoxide (DMSO).

Monocyte-derived dendritic cell isolation

Adherent cells remaining in the six-well plates were washed. GM-CSF (500 U/ml) and IL-4 (8 ng/ml) were added to each well (six days at 37°C, 5% CO_2) for the generation of monocyte-derived dendritic cells (moDC). Resulting cells were collected and irradiated to prevent moDC proliferation. moDC obtained in this manner were >93% pure and consisted of immature, CD11c+ HLA-DR+ $CD80^{low}$ $CD86^{low}$ dendritic cells.

Determining responses of lymphocytes and moDC to CVT-E002, with or without viruses

Lymphocytes were thawed, washed with 10% FBS in RPMI and added to 96-well plates (200 000 cells/well). Autologous moDC were added to the 96-well plates (5000 moDC/well) after washing. Plates containing various combinations of cells (lymphocytes alone, moDC alone, or lymphocytes with moDC) were incubated overnight, in the presence or absence of 100 or 500 µg/ml CVT-E002. The next day, human respiratory syncytial virus (RSV, type A2, ATCC, 18 000 pfu/well) or parainfluenza virus (PIV type 1, ATCC, 10^3 50% tissue culture infectious dose ($TCID_{50}$)) was added to the wells. UV-inactivated virus (15 min on ice, 254 nm 8 W lamp located 5 cm above the tube containing the virus) were used for some experiments. Co-cultures were incubated for seven days (37°C 5%CO_2) to allow lymphocyte activation. For this project, we used different high-incidence, common airway viruses: RSV and PIV. These viruses were chosen because they are ssRNA airway viruses that frequently infect humans throughout their lives. Since RSV and PIV are common airway infection agents, our donors were expected to have immunity to this virus in the form of memory T cells. To determine the role of protein in the CVT-E002 extracts, we inactivated using three treatments: heating at 95°C (h), trypsinization (t) or addition of 2-mercaptoethanol (m) for 5 min. Lipopolysaccharide (LPS; 10 ng/ml; St Louis, MO, USA) was used as a stimulation control for most experiments.

Characterization of leucocytes by flow cytometry and BrdU incorporation

To evaluate potential antigen-presenting cell function, the moDC were fixed with 5% formaldehyde for 5 min, blocked overnight with 0.5% bovine serum albumin (BSA) and incubated with PE-labelled anti-HLA-DR, FITC-labelled anti-CD80, PE-labelled anti-CD86, as well as APC-labelled anti-CD11c antibodies). Cells were gated on the CD11c+ population (>93%), using FACS Canto (BD Pharmingen). To evaluate T-cell activation after the various co-cultures were completed, cells were incubated with PerCP-labelled anti-CD3, Alexa488-labelled anti-CD4, PE-labelled anti-CD45RO, PE-labelled anti-CD8 and APC-labelled anti-CD25 antibodies and fixed with 1% paraformaldehyde. Results were analysed using WinMDI 2.8 and FACS Diva software. T-cell proliferation was measured using a BrdU (5-bromo-2′-deoxyuridine) Cell Proliferation ELISA chemiluminescent kit

(Roche, Mannheim, Germany), using FLx800 luminometer (Biotek, Winooski, VT, USA); proliferation was determined as relative light units (rlU/s).

Cytokine release measurement of supernatants from co-cultures of lymphocytes and moDC

Supernatants were collected from cell co-cultures after seven days of exposure to CVT-E002, with or without virus. Levels of IFN-γ, IL-12p70 and TNF were measured using Searchlight Protein Array Sample Testing Service (Aushon Biosystems, formerly part of Pierce Biotechnology, Billerica, MA).

TLR-4 and TLR-2 inhibition assays

Escherichia coli LPS (Sigma, St Louis, MO, USA) at 10 ng/ml was used as TLR-4 agonist. moDC were exposed to 10 μg/ml RS-LPS for 1 h at 37°C before adding CVT-E002 or the LPS for a further 24 h. For TLR-2 inhibition assays, moDC were treated with 10 μg/ml of an anti-TLR2 antibody (Clone 11G7, BD Biosciences, Mississauga, Ontario, Canada). Before CVT-E002 exposure; the synthetic lipopeptide palmitoyl-Cys((*RS*)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys-Lys-OH (Pam3CSK4, EMC microcollections, Tuebingen, Germany) was used as a selective agonist for TLR-2.

Statistical analysis

Each value of *n* represents one human donor, whose cells were aliquoted for each experimental condition. Flow cytometry and cytokine data from each treatment condition were pooled to represent each treatment group. BrdU data for T-cell proliferation was measured in triplicate, and the results pooled for each treatment. Data were analysed by Kruskal-Wallis test with Dunn correction to identify statistical differences between means, using GraphPadPrism version 4.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

CVT-E002 induced increased T-cell activation and proliferation through moDC

T-cells were cultured in the presence or absence of CVT-E002 treated or untreated moDC. T cell activation was quantified by the degree of surface CD25 expression (IL-2 receptor) and T cell incorporation of BrdU. In the absence of moDC, CVT-E002 did not induce T cell activation, as CD25 expression and T cell proliferation were similar to control (Figure 1). In contrast, when moDC were added to the co-culture, CVT-E002 induced increased CD25 expression and T cell proliferation compared with T cells and moDC without CVT-E002 (Figure 1a, c). Gating for CD3+CD4+ and CD3+CD8+ populations showed that the majority of T cells responding to the CVT-E002 treated moDC were CD4 positive (Figure 1a, b). There was a small increase in CD25 expression in the CD8+ T cells, which was not significant. We further determined that T cells activated by CVT-E002 had a memory phenotype (CD3+CD4+CD45RO+) (Figure 2a and b). Phytohemagglutinin (PHA) or LPS were used in place of CVT-E002 for all experiments as a positive control for T cell activation in absence or presence of dendritic cells, respectively.

CVT-E002 induced an increase in antiviral cytokine levels in T cell and moDC co-cultures before virus was present

We analysed the cytokine release profile of CVT-E002 treated cells for release of IFN-γ, IL-12p70 and TNF (Figure 3a).

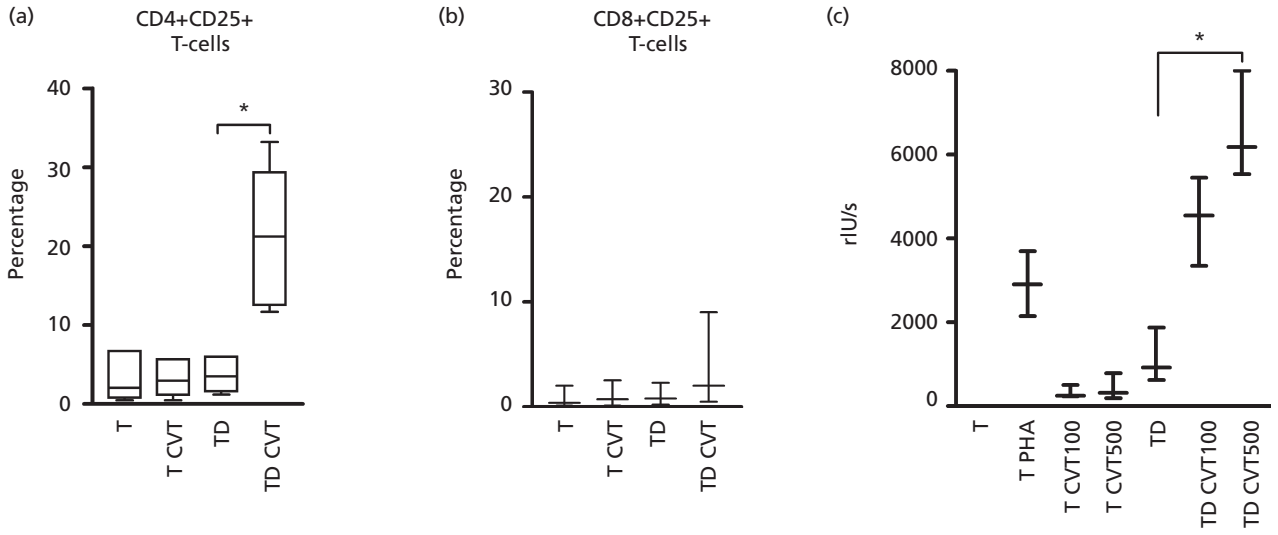

Figure 1 CVT-E002 induced activation of T cells when co-cultured with monocyte-derived dendritic cells. T cells were exposed to CVT-E002 (a and b, 500 μg/ml; c, 100 or 500 μg/ml) with or without monocyte-derived dendritic cells (moDC). CVT-E002 activated CD4+ T cells (a) but not CD8+ T cells (b) when co-cultured with moDC as determined by CD25 expression ($n = 5$). Numbers are percentages of the parent gated population (CD3+CD4+ or CD3+CD8+, respectively). (c) T cell proliferation was induced in a dose–response fashion by CVT-E002 in the presence of moDC (BrdU Chemiluminescent ELISA was measured in relative light units, rlU/s; $n = 3$). T, T cells; D, moDC; CVT, CVT-E002. Box plots are shown, $^*P < 0.05$.

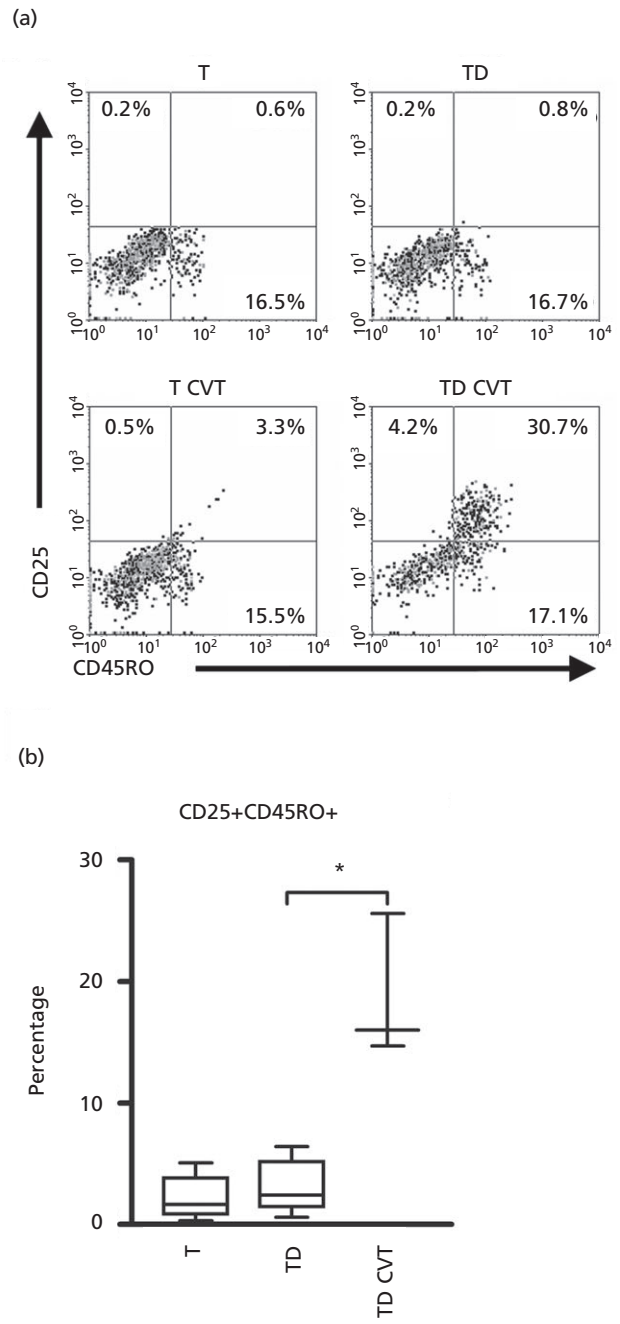

Figure 2 CVT-E002 induced activation of CD4+CD45RO+ memory T cells when co-cultured with monocyte-derived dendritic cells. T cells cultured with CVT-E002 (500 μg/ml) with or without monocyte-derived dendritic cells (moDC). (a) Representative flow cytometry density plot gated on the CD3+CD4+ cells. (b) Percentage of cells positive for CD45RO and CD25 in the CD3+ CD4+ gated population. T, T cells; D, moDC; CVT, CVT-E002. Box plots are shown. $*P < 0.05$; $n = 4–8$.

Similar to the T cell activation data, CVT-E002 had no ability to induce T cell cytokine production without moDC (not shown). In contrast, when moDC were added to T cells, CVT-E002 treatment increased the levels of all three cytokines in the supernatants compared with T cells with untreated moDC.

CVT-E002 appeared to increase T cell responses to virus

We measured the levels of T cell CD25 expression and cytokine release in the presence of airway viruses. RSV induced release of all three cytokines from T cells co-cultured with moDC (Figure 3a). Without moDC there was no cytokine production noted (data not shown). While the combination of both CVT-E002 and RSV caused a further increase in release for each cytokine compared with RSV alone, given the variance in the data, statistical significance was not reached. We also looked at our co-cultures of T cells and moDC exposed to PIV, with or without CVT-E002. As might be expected, the expression of CD25 on T-cells did increase in response to PIV when moDC were present (data without moDC showed no increase, not shown). The addition of CVT-E002 to moDC with PIV showed further increase in CD25 expression compared with moDC with virus alone (Figure 3b). The variance for the expression of CD25 was less in these experiments, as such, this comparison did reach statistical significance.

CVT-E002 induced increased expression of markers of maturation on moDC

To determine the mechanism of increased T cell activation through moDC, we measured cell surface markers to characterize the maturation state of CVT-E002-treated moDC. The mean fluorescence intensity (MFI) of the moDC maturation marker CD83, as well as the co-stimulatory molecules CD80 and CD86 were all increased in a dose–response fashion in CVT-E002-treated moDC (100 and 500 μg/ml) compared with sham-treated cells (Figure 4). While there was a trend to increased expression of major histocompatibility complex (MHC) class II (HLA-DR), these differences did not reach statistical significance. CVT-E002 treatment also increased the number of moDC expressing CD86 and CD80 (Figure 5), which appeared to be dose-dependent. LPS (10 ng/ml) was used as a positive control for moDC maturation (data not shown).

The active component of CVT-E002 was likely not a protein

We sought to identify which of the different components of CVT-E002 was inducing moDC maturation and ultimately T cell activation. CVT-E002 is a polysaccharide-enriched fraction of *P. quinquefolium* which contains 70% carbohydrate, 20% uronic acid – which is derived from carbohydrates – and 10% protein, water and other as yet unknown compounds.[5] We inactivated the protein component of CVT-E002 by heat (95°C), trypsin or 2-mercaptoethanol treatment for 5 min immediately before addition to our moDC cultures. The ability of CVT-E002 to induce increased CD80 or CD86 expression was unaltered by these treatments, making it unlikely that the active component of CVT-E002 was a protein (Table 1).

CVT-E002 induced maturation of moDC was not mediated by LPS or TLR-4 signalling

We considered the role of pattern recognition receptors signalling in moDC activation and the possibility that CVT-E002 mimicked LPS-induced moDC maturation. Using a

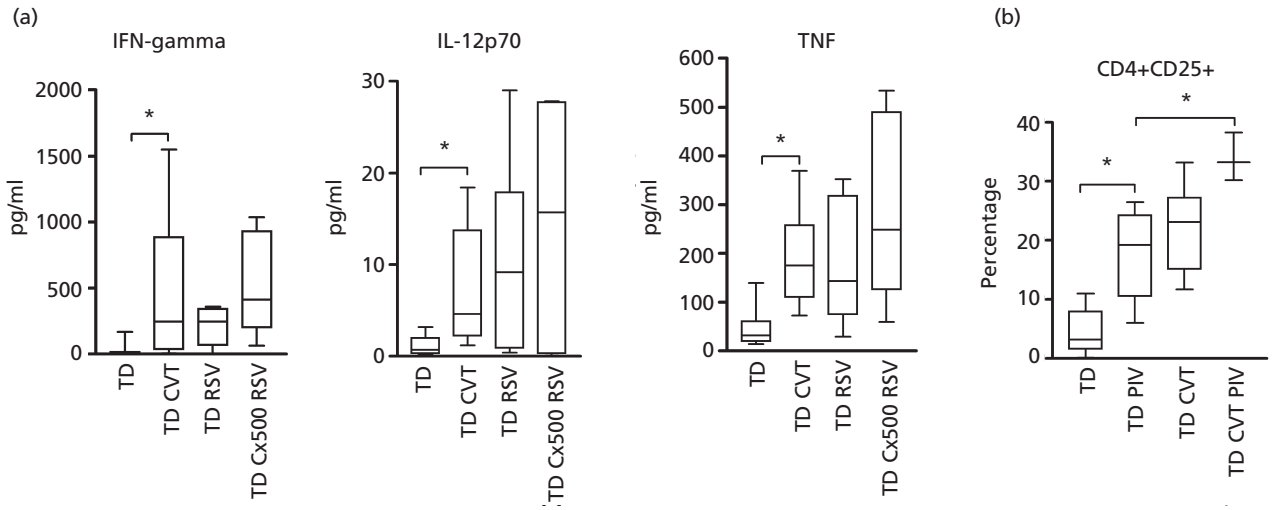

Figure 3 The response of T cell and monocyte-derived dendritic cell co-cultures to CVT-E002 with or without virus. T cells with monocyte-derived dendritic cells (moDC) were cultured with or without CVT-E002 (500 μg/ml) in the presence or absence of virus, either respiratory syncytial virus (RSV) or parainfluenza virus (PIV). (a) The level of cytokines interferon-γ (IFN-γ; $n = 8–10$), interleukin-12p70 (IL-12p70; $n = 7–8$), and tumour necrosis factor (TNF; $n = 5–9$) measured by ELISA. (b) The percentage of CD25+ cells in CD4+ T populations measured by flow cytometry ($n = 3–11$). T, T cells; D, moDC; CVT, CVT-E002. Box plots are shown. $^*P < 0.05$.

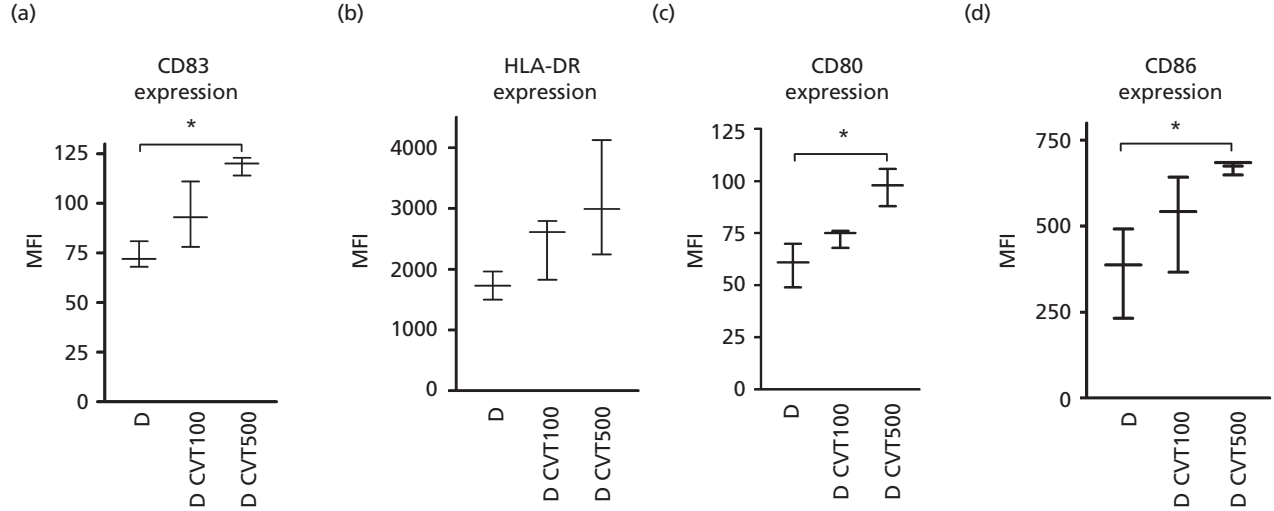

Figure 4 CVT-E002 induced increased maturation of monocyte-derived dendritic cells. Mean fluorescence intensity (MFI) of maturation markers (a) CD83, (b) HLA-DR, and co-stimulatory molecules (c) CD80, (d) CD86 were determined by flow cytometry in CD11c+ cells with or without CVT-E002 100 μg/ml (CVT100) or 500 μg/ml (CVT500). D, monocyte-derived dendritic cells. Box plots are shown. $^*P < 0.05$. a and b, $n = 3$; c and d, $n = 5$ each.

Table 1 The CVT-E002 factor that induced monocyte-derived dendritic cell maturation was not a protein

	Dendritic cells alone	Dendritic cells plus CVT-E002	Dendritic cells hCVT-E002	Dendritic cells tCVT-E002	Dendritic cells mCVT-E002
Mean expression of CD80	23.39	41.89	41.76	40.30	40.73
Standard deviation	6.169	4.960	7.362	6.443	4.970
Standard error	2.181	1.754	2.783	2.435	2.029

We inactivated CVT-E002 by heat (h, 95°C), trypsin (t) or 2-mercaptoethanol (m) treatment immediately before addition to our monocyte-derived dendritic cell cultures ($n = 3$ each). The ability of CVT-E002 to induce increased CD80 % expression was unaltered by these treatments.

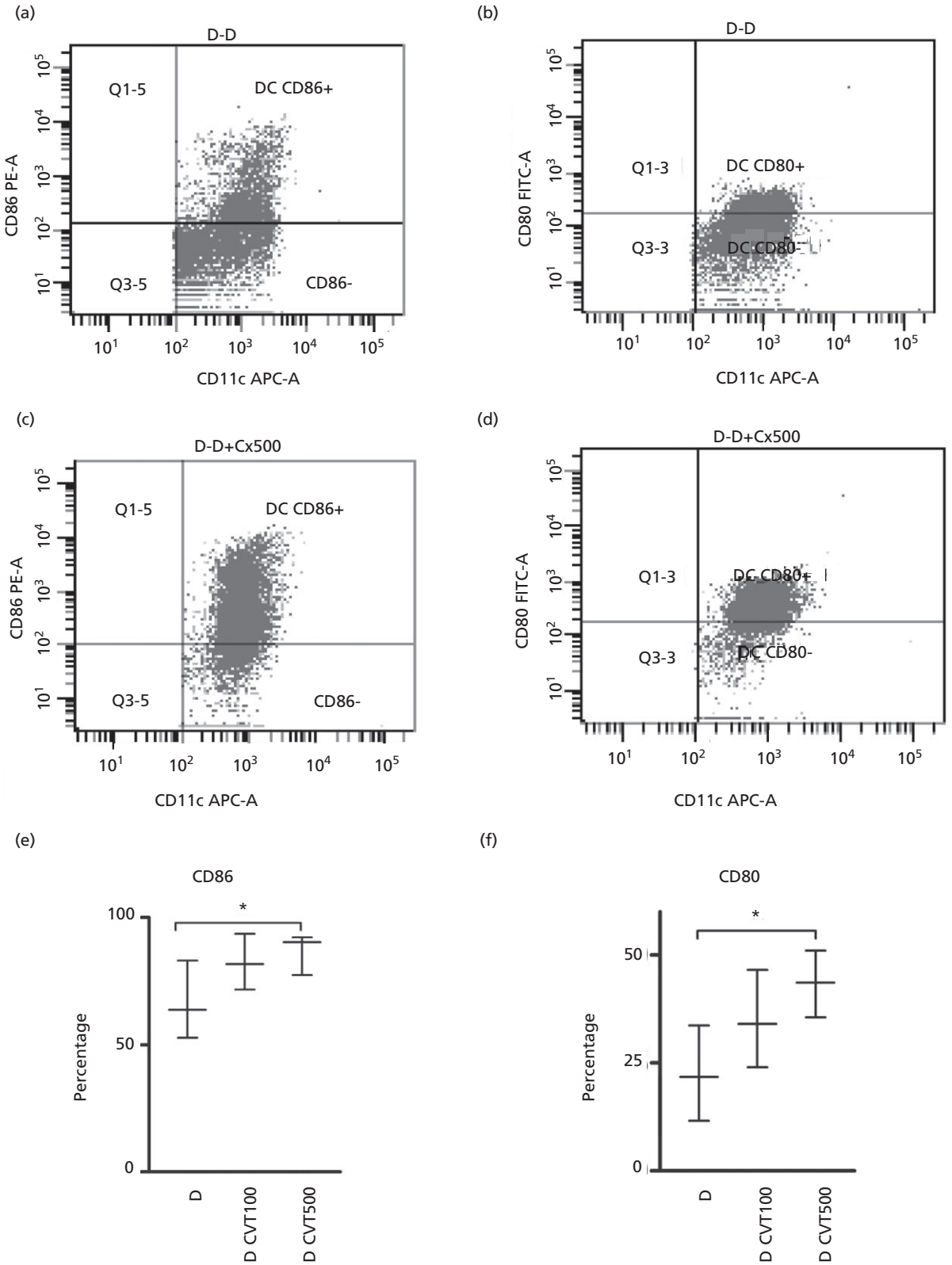

Figure 5 CVT-E002 induced an increased number of CD80+ and CD86+ monocyte-derived dendritic cells. The percentage of CD80 and CD86 positive cells was determined by flow cytometry in the CD11c parent population of monocyte-derived dendritic cells (moDC) that were untreated or exposed to CVT-E002 100 (CVT100) or 500 (CVT500) μg/ml. Representative dot plots are shown for expression of CD86 and CD80 in moDC without CVT-E002 (a and b, respectively) and with CVT-E002 500 μg/ml (c and d, respectively). Box plots shown correspond to all data for CD86 (e) and CD80 (f); $n = 5$ each, $^*P < 0.05$.

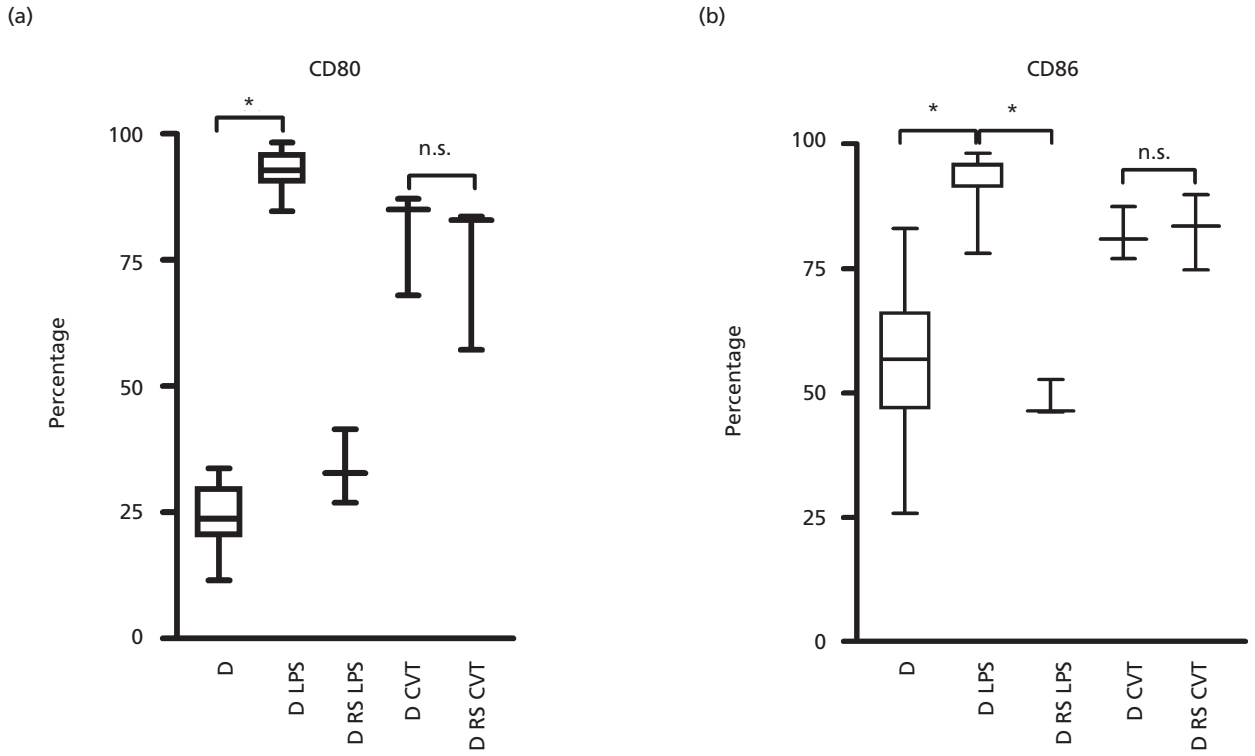

Figure 6 CVT-E002 induced maturation of monocyte-derived dendritic cells was not mediated by Toll-like receptor-4. Monocyte-derived dendritic cells (moDC) were incubated for 1 h with Toll-like receptor-4 (TLR-4) antagonist *Rhodobacter sphaeroides* lipopolysachharide (RS, 10 μg/ml) before exposure to either lipopolysaccharide (LPS, 100 ng/ml) or CVT-E002 (CVT, 500 μg/ml) for 24 h. Expression levels of CD80 (a) and CD86 (b) were determined in CD11c+ cells. Box plots are shown. $*P < 0.05$; n.s., no significant differences; $n = 3$ each.

commercially available kit (E-TOXATE *Limulus* Amebocyte Lysate test, Sigma, St Louis, MO, USA), LPS content in the CVT-E002 samples was negligible (<0.05 endotoxin U/ml, or approximately 5 pg/ml). We attempted to block the CVT-E002 effect using a known competitive antagonist of TLR-4, RS-LPS.[12] Despite its ability to block the effect of LPS, we found that the increased expression of CD80, or CD86 was unchanged by the presence of RS-LPS in CVT-E002 exposed moDC (Figure 6).

Discussion

In Chinese medicine, Ginseng extracts have been used for many years to improve health and prevent infection.[13] Unlike other available Ginseng extracts, CVT-E002 is purified. CVT-E002 contains no detectable LPS and only trace ginsenosides, which have been related to unwanted side effects.[14–17] In this report, we have provided in-vitro evidence that, while having no discernable effect on human T cells directly, there is strong induction of T cell activation in response to CVT-E002 only when moDC are added to the co-culture. In keeping with this, it appeared that the CD4+, and not CD8+ T cells were the populations activated by CVT-E002, and more specifically those with a CD45RO+ memory phenotype.

In keeping with our hypothesis, we found that CVT-E002 enhanced the induction of Th1-type cytokines typical of an antiviral response even before virus was added.[18] IFN-γ is a multi-functional Th1-type cytokine that induces cell-based antiviral immunity while inhibiting Th2 allergic responses. While TNF alone has its own plethora of immune effects, synergistic interactions between IFN-γ and TNF potentiate their antiviral properties, inhibiting viral gene expression and replication to a greater extent when both cytokines are combined.[19] As for IL-12, its heterodimeric active form (IL-12p70) is known to induce CD4+ T lymphocytes to synthesize interferons through activation of IRF-1.[20,21] The enhanced release of these cytokines by CVT-E002 even before virus was added to the co-culture, provided insight into the mechanism of the previously described role of CVT-E002 as an antiviral supplement in humans.[7,8]

There are other factors important in viral inhibition (i.e. IFN-α or CD8+ T cells), but typically these occur through recognition of virus infection (i.e. MHC-I). Our model was not designed to test this, as we did not have an infected epithelial cell in co-culture. Further, the method we used to culture our dendritic cells should not create plasmacytoid dendritic cells, which are known for IFN-α production. While there is the potential that such antiviral responses could have side effects, these have not been reported and the safety of CVT-E002 has been well documented in animals, adults and children.[5–7]

Our data suggested that CVT-E002 acted through augmented antigen-presentation. Dendritic cells play a key role in fighting viral infections, having the strongest ability to present antigens and induce T-cell mediated immunity.[22] Immature dendritic cells, characterized by low cell surface expression of

co-stimulatory molecules (CD80, CD86), are highly phagocytic but do not induce strong T-cell activation.[10,23] Upon stimulation, typically through pattern recognition receptors, dendritic cells mature and upregulate the expression of co-stimulatory molecules CD80 and CD86.[24] CD83 is up-regulated in mature moDC and appears to be important for activation and maturation of lymphocytes, but its actual function has not as yet been determined.[25] Without co-stimulation, T cells that recognize MHC-antigen complexes go into anergy rather than a state of activation.[26] In our experiments, CVT-E002 induced upregulation of CD80 and CD86 on the surface of the moDC, as well as that of CD83. CD80 and CD86 have different affinities for their co-stimulatory T cell counterparts. CD80 binds CTLA-4 with higher affinity than CD28, while CD86 is the opposite.[27,28] While CD28 co-stimulation induces T-cell activation and proliferation, CTLA-4 is inhibitory.[26,27,29,30] While the HLA-DR expression data did not reach statistical significance, there was a trend to increased expression. It is important to note that this method of isolating moDC created high levels of HLA-DR expression even before seeing an antigen, thus it would be more difficult to demonstrate a statistically significant difference for the relatively small number of experiments performed.

Overall, activation of T cells occurred even before the addition of virus. This suggested that part of the CVT-E002 effect was not antigen specific. This did not detract from our results as even a nonspecific activation of Th-1 cytokines could be preventative for virus infection. After adding virus we saw further increase in T cell activation, in particular, memory CD4+ T cells, and a trend to further increase in cytokine release. We suggest that improved moDC maturation and ability to induce T cell activation in a Th-1-fashion is a plausible mechanism for CVT-E002 antiviral properties. CVT-E002 may also have been maintaining a T cell pool that was more active, which could respond faster when a virus was found. Memory CD4+ T cells promoted enhanced responses from CD8+ and natural killer T cells, and also released cytokines that had antiviral properties.[31] They play a role in antigen-specific dendritic cell licensing needed for the establishment of memory and effector cytotoxic lymphocyte responses.[32]

Several mechanisms have been proposed for the induction of maturation of dendritic cells, as seen by increased CD80 or CD86 expression.[33,34] Amongst these is the pathogen-associated molecular patterns and pattern recognition receptors, including Toll-like receptors (TLR). Stimulation of TLR-4 by LPS, or TLR-1 and TLR-2 by bacterial signals such as peptidoglycan, leads to dendritic cell maturation and activation.[35–40] The biochemical composition of CVT-E002 is mainly a polysaccharide and could constitute a molecule with a pathogen-associated molecular pattern-like structure.[5] LPS was not detected in CVT-E002 and the use of TLR-4 inhibitor RS-LPS did not reduce the effect of CVT-E002 on the expression of co-stimulatory molecules CD80 or CD86 on moDC. Thus, we believe we have ruled out the role of LPS or LPS-like molecules acting through TLR-4 signalling for the induction of maturation of moDC, at least through canonical activation. Dendritic cells have carbohydrate receptors on their surface, typically used to sense the presence of bacteria. Among these receptors, lectin receptors might play a relevant role in CVT-E002-mediated dendritic cell activation.

We considered other pattern recognition receptors as well. TLR-2 signalling is an inducer of dendritic cell maturation and activation.[37,40] We attempted to identify a role for TLR-2 in our system using the commercially available anti-TLR-2 antibody (clone 11G7, BD Biosciences). This clone was reported to block TLR-2 stimulation by ara-lipoarabinomannan or the synthetic lipopeptide Pam3CSK4, but not zymosan.[41] Unfortunately, in our system we could not replicate these data as the induction of CD80 or CD86 by Pam3CSK4 remained uninhibited (data not shown). Thus, we could not confirm a role for TLR-2 signalling in the CVT-E002 stimulatory effect. Our protein denaturing data suggested that the active component of CVT-E002 was likely to be a carbohydrate and not a protein. Other pattern recognition receptors could be potential targets for such a carbohydrate stimulus, hence it would be interesting to assess this in the future.[42,43]

Conclusions

To date, the components of Ginseng extracts, as well as the mechanisms contributing to their purported beneficial effects have not been vigorously tested using human leucocytes. This is the first report that has shown that CVT-E002 did stimulate an antiviral immune response in human leucocytes. It appeared that CVT-E002 acted on moDC to enhance T cell Th1-like responses. Our data supported the hypothesis that CVT-E002 could act as an immune modulator for the prevention of viral airway infections. Further, we suggest that our data will be relevant in the design of future studies for the prevention of virus-induced exacerbation of chronic airways diseases such as asthma or chronic obstructive pulmonary disease.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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