Article

Modulation of TRPV1 function in human CD4+ T cells by nanodiamond and nanoplatinum liquid, DPV576

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Abstract: Transient receptor potential vanilloid (TRPV) channels act as sensors of pain, temperature, and other external stimuli. We have recently shown that DPV576, an aqueous mixture of nanodiamond (ND) and nanoplatinum (NP), can modulate the activity of TRPV on human primary keratinocytes, suggesting their potential as a possible pain modulator [1]. CD4+ T lymphocytes also express TRPV channels, and we sought with special interest to examine the effect of DPV576 in modulating the functions of TRPV channel expression and secretion of cytokines on human CD4+ T lymphocytes. Human primary CD4+ T cells were activated with anti CD3/CD28 with and without DPV576 at 1:25 and 1:100 dilutions for 24 hours *in vitro*. TRPV Receptor expression (TRPV1 and TRPV4) on CD4+ T cells was examined by flow cytometry. The capacity of DPV576 to modulate the activity of TRPV1 agonist capsaicin in CD4+ T cells was also determined. Activation of CD4+ T cells was determined by production of cytokines TNF-α, IFN-γ, and IL-10 using specific ELISA kits. DPV576 treatment of CD4+ T cells that were activated with anti CD3/CD28, resulted in increased expression of TRPV1 channel but had no effect on TRPV4. This was accompanied by increased secretion of IFN-γ and reduced expression of TRPV1 in capsaicin activated CD4+ T cells. In addition, DPV576 inhibited the capsaicin, induced the production of both IFN-γ and TNF-α, and enhanced the secretion of IL-10. We conclude that short term exposure to DPV576 inhibits the activity of TRPV1 channels in CD4+ T lymphocytes, which may suggest its possible beneficial use for pain management.

Keywords: DPV576; CD4+ T cells; TRPV1; capsaicin

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1. Introduction

Tissue damage, due to noxious physical, chemical, or mechanical stimuli, constitutes a major challenge to homeostasis, which disrupts tissue integrity and triggers a coordinated and diverse response mediated by effector cells such as immune cells, peripheral neurons, fibroblasts, and endothelial cells. A hallmark feature of such damage is the release of inflammatory mediators that activate receptors leading to altered cellular activities. Many of these mediators bind to receptors expressed on afferent terminals, leading to dramatic changes in neuronal excitability [2]. For example, human dental pulp and trigeminal ganglion (TG) neurons express the TLR-4 receptor, and application of bacterial endotoxins directly activates and sensitizes TG neurons, providing a mechanism for pain due to orofacial infections [3].

The family of transient receptor potential (TRP) is an evolutionarily conserved group of ligand-gated ion channels that respond to a variety of stimuli, including variations in temperature, pH, osmolarity, pro-inflammatory agents, and multiple endogenous or exogenous stress mediators [4]. Our work and that of others showed that members of the TRP family are expressed in a wide variety of different cell types such as neurons, skin, mesenchymal stem cells, vascular cells, fibroblasts, and immune cells [1, 5-7]. TRP channels have been studied as pain receptors [8].

Immune cells are major mediators of inflammation and inflammatory cytokines which act on the neurons to induce pain. Inflammatory cytokines are also considered major fatigue and stress inducers. Earlier studies reported expression of the transient receptor potential vanilloid (TRPV) receptors in human blood lymphocytes [9], in normal human T lymphocytes [10], and in CD4+ T cells [11-12]. Earlier studies have reported the expression of several TRP channels in rodent and human T cells [15-17]. The TRPV family can be divided into two subfamilies: the first subfamily comprises TRPV1-4, acts as a non-store operated Ca2+ channel [11], and is known to participate in thermosensation [4]; the other subfamily comprises TRPV5/6, is exclusively permeable to Ca^{2+}, and is viewed as the gatekeeper of epithelial calcium transport [5, 18-19]. It has been shown that genetic deletion or pharmacological inhibition of TRPV1 in $CD4^+$ T cells substantially reduced colitis severity in animal models of human inflammatory bowel disease [11]. These data suggest that targeting the TRPV channel could represent a novel strategy to inhibit pro-inflammatory $CD4^+$ T cell responses in related human diseases.

Therefore, compounds aimed at decreasing the expression of TRP channels are emerging as major painkillers. An example is capsaicin I-RTX (a specific TRPV1 antagonist) [13]. We have recently shown that a dispersed aqueous mixture of nanodiamond (ND) and nanoplatinum (NP), DPV576, can down regulate the expression of TRPV4 and, to a certain extent, TRPV1 on human primary keratinocytes, suggesting its potential as a possible pain modulator [1]. The current study was carried out to further expand the potential role of DPV576 on inflammatory responses induced by TRPV activation on CD4+ T lymphocytes. These results have the potential to facilitate the design of future combinations of ND and NP therapeutics aimed at modulating local inflammation and pain. To our knowledge, this study is the first investigation into these potential therapeutic properties of nanoparticles.

2. Materials and Methods

2.1 Blood donors.

Peripheral blood samples were obtained from healthy volunteers under an IRB approved protocol.

2.2 DPV576 liquid.

An aqueous mixture of nanodiamond (ND) and nanoplatinum (NP) mixture solution known as DPV576 was used [13]. We have previously described the details regarding particle size, shape, and composition of ND and NP mixture [13]. DPV576 was supplied by Venex Co, Ltd, Kanagawa, Japan.

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2.3 CD4+T cell purification.

CD4+ T cells were purified from the PBMCs by negative selection using CD4+ T cell enrichment kit (Stemcell technologies, Vancouver), with purity above 90%.

Effect of DPV576 on T cells activation. Purified CD4+ T cells were stimulated with anti-CD3 and anti-CD28 beads (STEMCELL Technologies) along with DPV576 concentrations of 1:25 and 1:100 for 24 hours. The expression of TRPV1, TRPV4 on the cells was determined using flow cytometry. Briefly, cells were collected and centrifuged, re-suspended in PBS (phosphate buffered saline) 2% FBS (fetal bovine serum), and incubated with specific antibodies for TRPV1 or TRPV4 (Bioss Inc, Woburn, MA) for 1 hour. Subsequently, the cells were washed and a minimum of 10,000 cells were acquired on FACS Calibur (Becton Dickinson, San Jose, CA). Isotype antibodies were used as controls. Analysis of the expression of TRPVs was performed by FlowJo (FlowJo, LLC, Ashland, OR). Supernatant collected were assayed for cytokines IFN-γ, TNF-α, and IL-10 using specific ELISA's (BD Biosciences).

2.4 Stimulation with anti-CD3 and anti-CD28 beads in the presence and absence of capsaicin and DPV576.

Purified CD4+ T cells were stimulated with anti-CD3 and anti-CD28 beads (STEMCELL Technologies) plus DPV576 concentrations of 1:25 and 1:100 for 24 hours in the presence or absence of capsaicin 10mM/ml (Sigma). Supernatants collected were assayed for cytokines IFN-γ, TNF-α, and IL-10 using specific ELISA's (BD Biosciences).

2.5 Statistical Analysis.

Statistical analysis for experiments was performed using GraphPad Prism (GraphPad Inc., San Diego, CA, USA). T-test was used for analysis. A *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1 DPV576 modulates the expression of TRPV1 on CD4+ T cells.

We determined the expression of TRPV1 channels on CD4+ T cells with and without DPV576 using flow cytometry. First, we examined the effect of DPV576 on expression of TRPV1 on unactivated CD4+ T cells. As shown in Figure 1, exposure of CD4+ T to DPV576 in the absence of T cell activation did not have a significant effect on the expression of TRPV1 on CD4+ T cells (Figure 1A, top panel).

Second, we examined the effect of DPV576 on expression of TRPV1 on CD4+ T cells activated with anti-CD3/CD28. When T cells were activated with anti-CD3 and anti-CD28 beads in the presence of DPV576, we observed upregulation of TRPV1 expression over control cells (Figure 1A, bottom panel). These data suggest that DPV576 modulates the expression of TRPV1 on activated CD4+ T cells.

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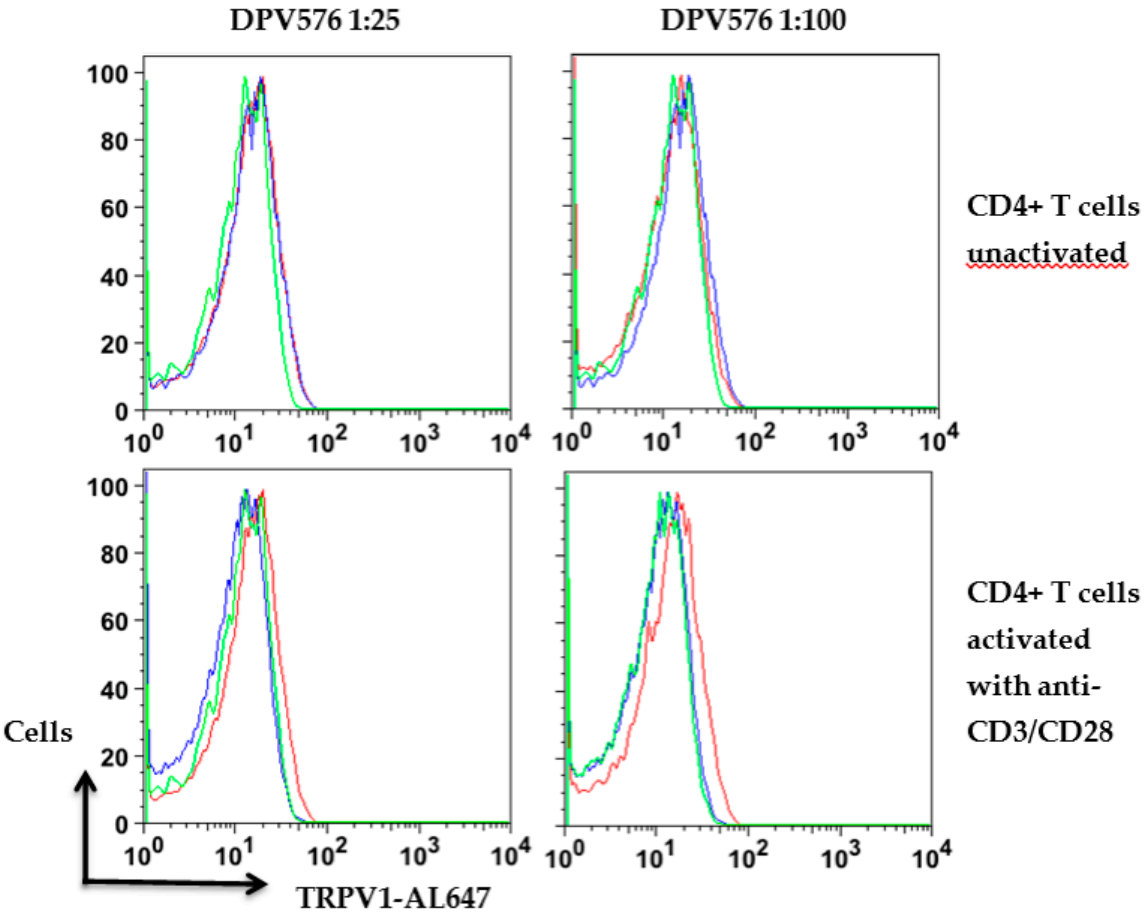

Figure 1. DPV576 modulates the expression of TRPV1 on CD4+ T cells. Unactivated and anti-CD3/CD28-activated CD4+ T lymphocytes were exposed to DPV576 for 24 hours. The cells were stained for TRPV channels. Expression of TRPV1 on: (a) Unactivated T cells (Top panel); (b) anti-CD3/CD28-activated T cells (bottom panel). Data is representative of 3 such experiments. Blue: without DPV576; Red: DPV576-exposed lymphocytes; Green: isotype.

3.2 *DPV576 does not modulate the expression of TRPV4 on CD4+ T cells.*

Next, we examined whether DPV576 also affected the expression of TRPV4 on CD4+ T cells. As shown in Figure 2, exposure of CD4+ T cells to DPV576 in the absence of T cell activation did not have a significant effect on the expression of TRPV4 on CD4+ T cells (Figure 2, top panel). Similarly, there was no significant effect on the expression of TRPV4 on anti-CD3/CD28-activated CD4+ T cells (Figure 2, bottom panel). These data suggest that DPV576 has no effect on the expression of TRPV4 on CD4+ T cells.

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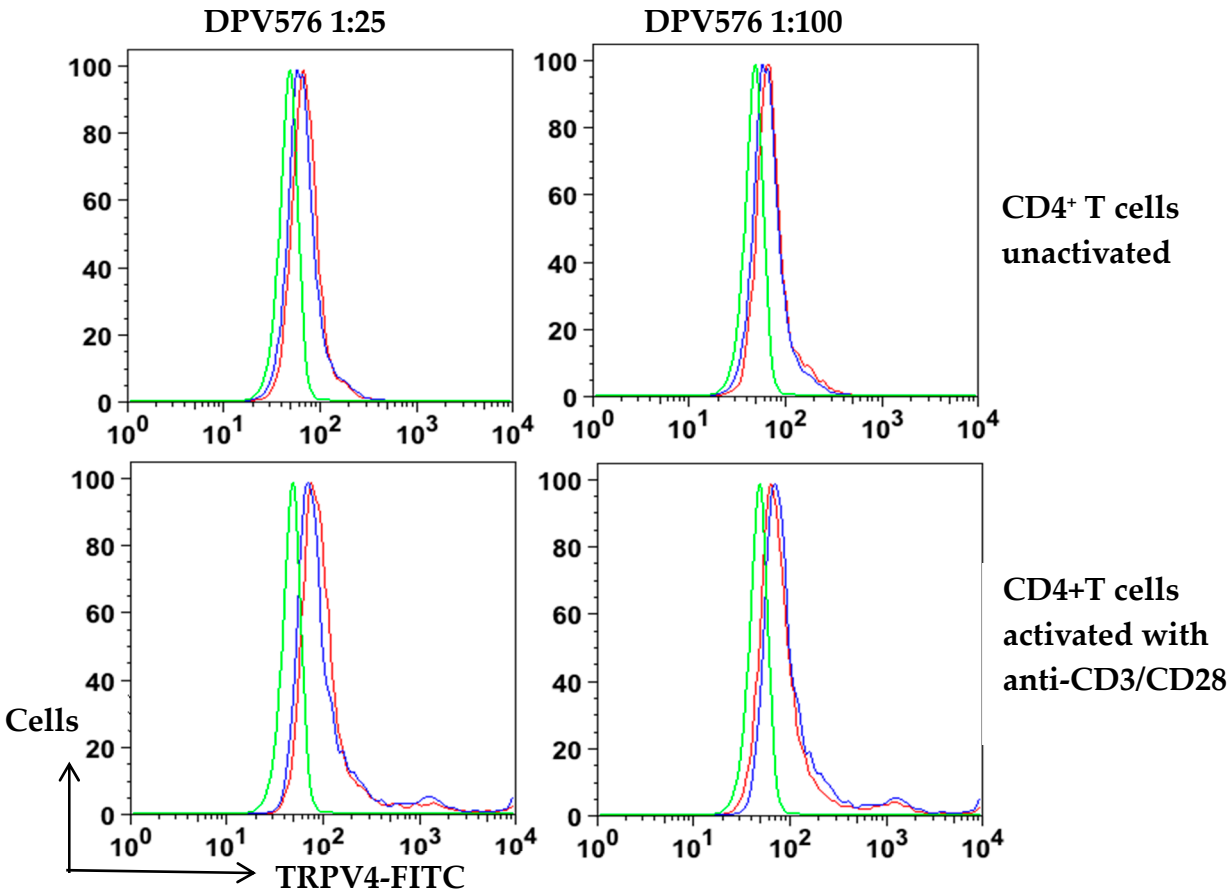

Figure 2. DPV576 modulates the expression of TRPV4 on CD4+ T cells. Unactivated and anti-CD3/CD28-activated CD4+ T lymphocytes were exposed to DPV576 for 24 hours. The cells were stained for TRPV channels. Expression of TRPV4 on: (A) Unactivated T cells (Top panel); (B) anti-CD3/CD28-activated T cells (bottom panel). Data is representative of 3 experiments. Blue: control; Red: DPV576 exposed lymphocytes; Green: isotype.

3.3 DPV576 enhances cytokine secretion from activated CD4+ T cells.

Cytokine secretion by T cells is a measure of their inflammatory activity; therefore, we examined the effect of DPV576 on cytokine secretion by CD4+ T cells. Data in Figure 3 shows that exposure of CD4+ T lymphocytes to DPV576 alone shows undetectable levels of cytokines IFN-γ, TNF-α, and IL-10 without activation. However, pre-activation of CD4+ T cells with anti-CD3 and anti-CD28 beads followed by exposure to DPV576 resulted in enhanced secretion of IFN-γ (Figure 3A). On the other hand, there was no induction of TNF-α or IL-10 over controls even in the activated CD4+ T cells (Figure 3B & C). These data suggest that DPV576 induces IFN-γ secretion in CD4+ T cells.

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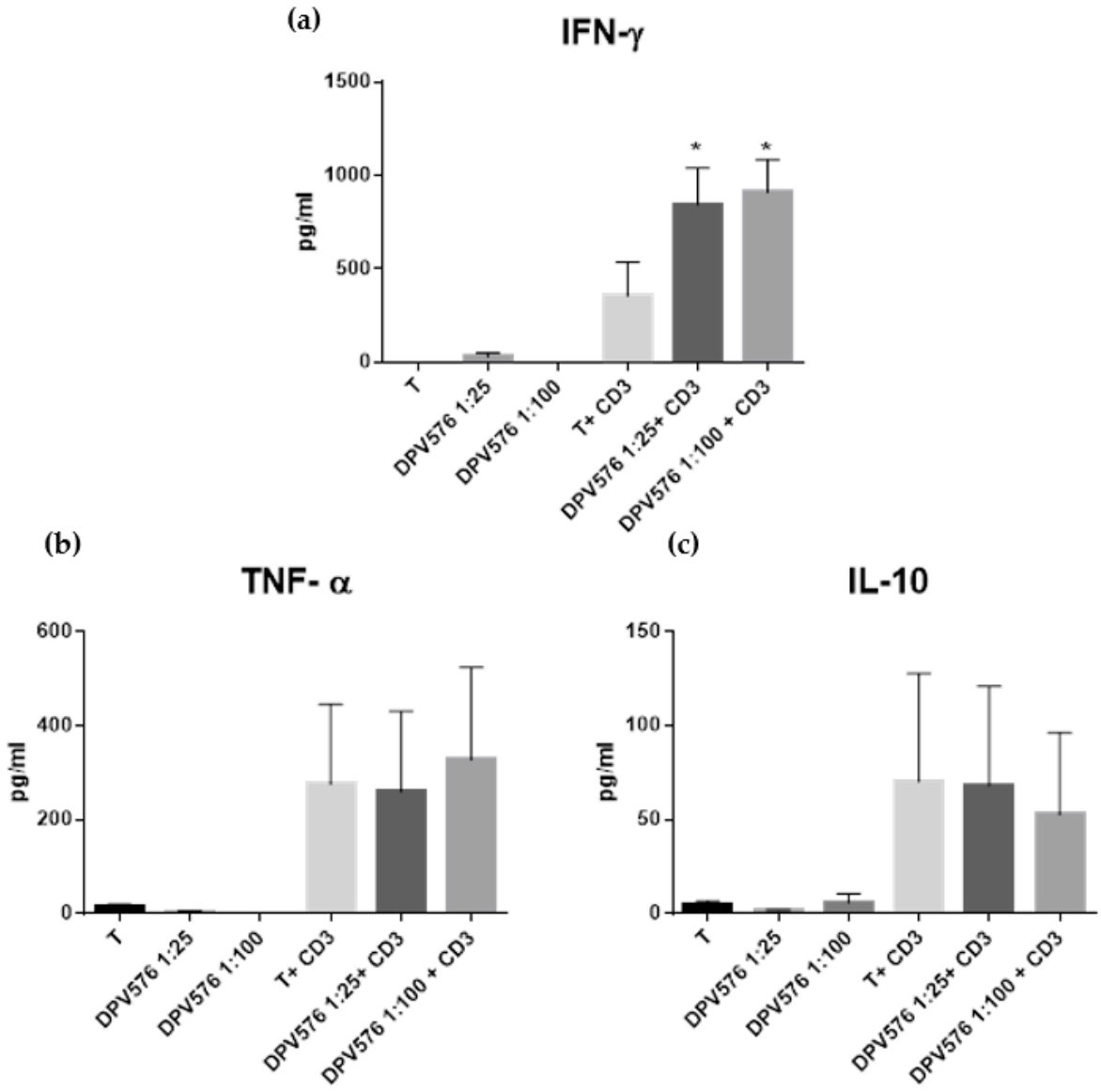

Figure 3. DPV576 induced cytokine secretion from CD4+ T cells. Unactivated and anti-CD3/CD2-activated CD4+ T lymphocytes were cultured in the presence of DPV576 for 24 hours. Secretion of cytokines was determined by ELISA. Bar graph depicts the level of: (a) IFN-γ; (b) TNF-α; (c) IL-10. Data is mean +/- S.E. of three experiments. * $p<0.05$.

3.4 DPV576 down modulates the capsaicin induced expression of TRPV1 on CD4+ T cells.

To investigate the effect of DPV576 on the TRPV1 agonist capsaicin-induced TRPV1 activity, we determined the expression of TRPV1 on anti-CD3- and anti-CD28-activated CD4+ T cells stimulated with capsaicin, in the presence or absence of DPV576. TRPV1 expression after 24 hours was determined by flow cytometry. Results suggest that capsaicin upregulates the expression of TRPV1 on CD4+ T cells, which is down regulated in the presence of DPV576 (Figure 4). Thus DPV576 may inhibit the activity of capsaicin in CD4+ T cells.

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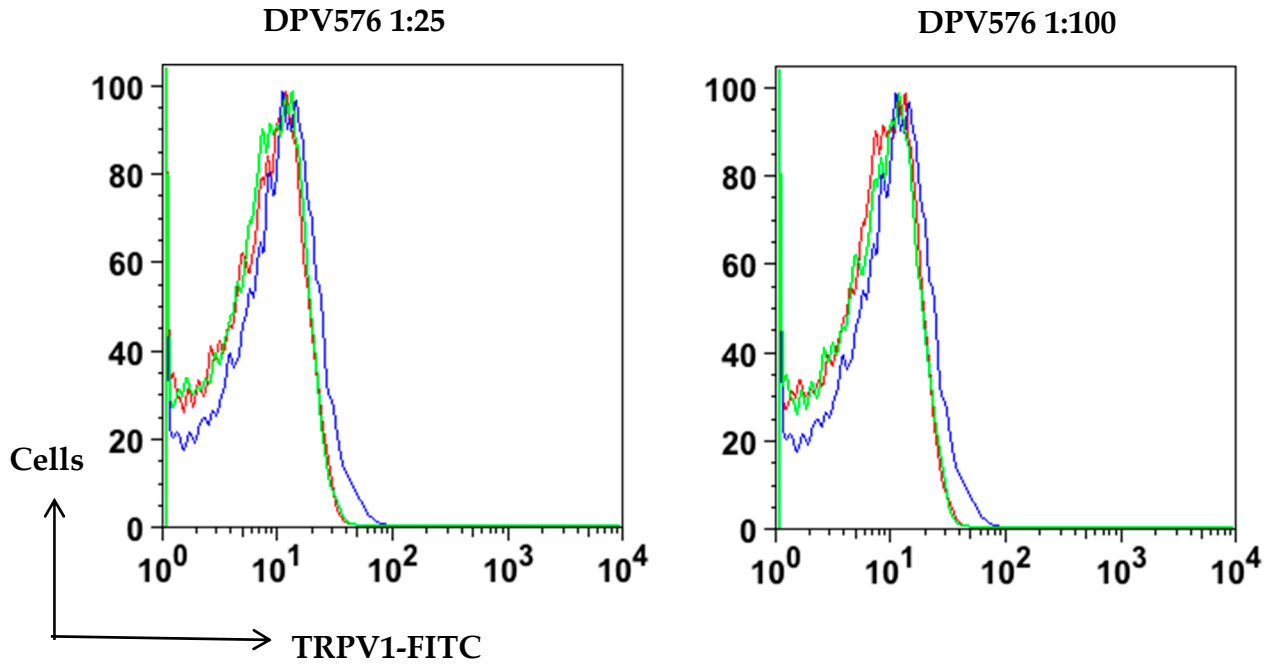

Figure 4. DPV576 down regulates the expression of TRPV1 in capsaicin-activated CD4+ T cells. Anti-CD3/CD28 + capsaicin activated CD4+ T lymphocytes in the presence of DPV576 for 24 hours. The cells were stained for TRPV1. Data is representative of 3 experiments. Blue: capsaicin; Red: capsaicin+ DPV576 exposed lymphocytes; Green: anti-CD3/CD28.

3.5 DPV576 modulates cytokine secretion of TRPV1 agonist capsaicin in CD4+ T cells.

Changes in temperature and pain are sensed by TRPV channels on T cells and can lead to their activation and cytokine secretion [11]. We observed a decrease in TRPV1 expression by DPV576 in the capsaicin-induced upregulation of TRPV1 in CD4+ T cells (Figure 4), which suggests an inhibition of capsaicin activity by DPV576. To investigate this possibility, anti-CD3- and anti-CD28-activated CD4+ T cells were stimulated with capsaicin in the presence or absence of DPV576. Cytokine secretion after 24 hours was determined by ELISA. Results in Figure 5 indicate that stimulation of CD4+ T cells with capsaicin alone leads to the secretion of IFN-γ and low levels of IL-10. The addition of DPV576 to capsaicin-primed CD4+ T cells caused the following: 1) inhibition of IFN-γ secretion, 2) increased production of IL-10, and 3) no effect on TNF-α production. These results indicate that DPV576 can modulate the activity of TRPV1 channels in CD4+ T cells.

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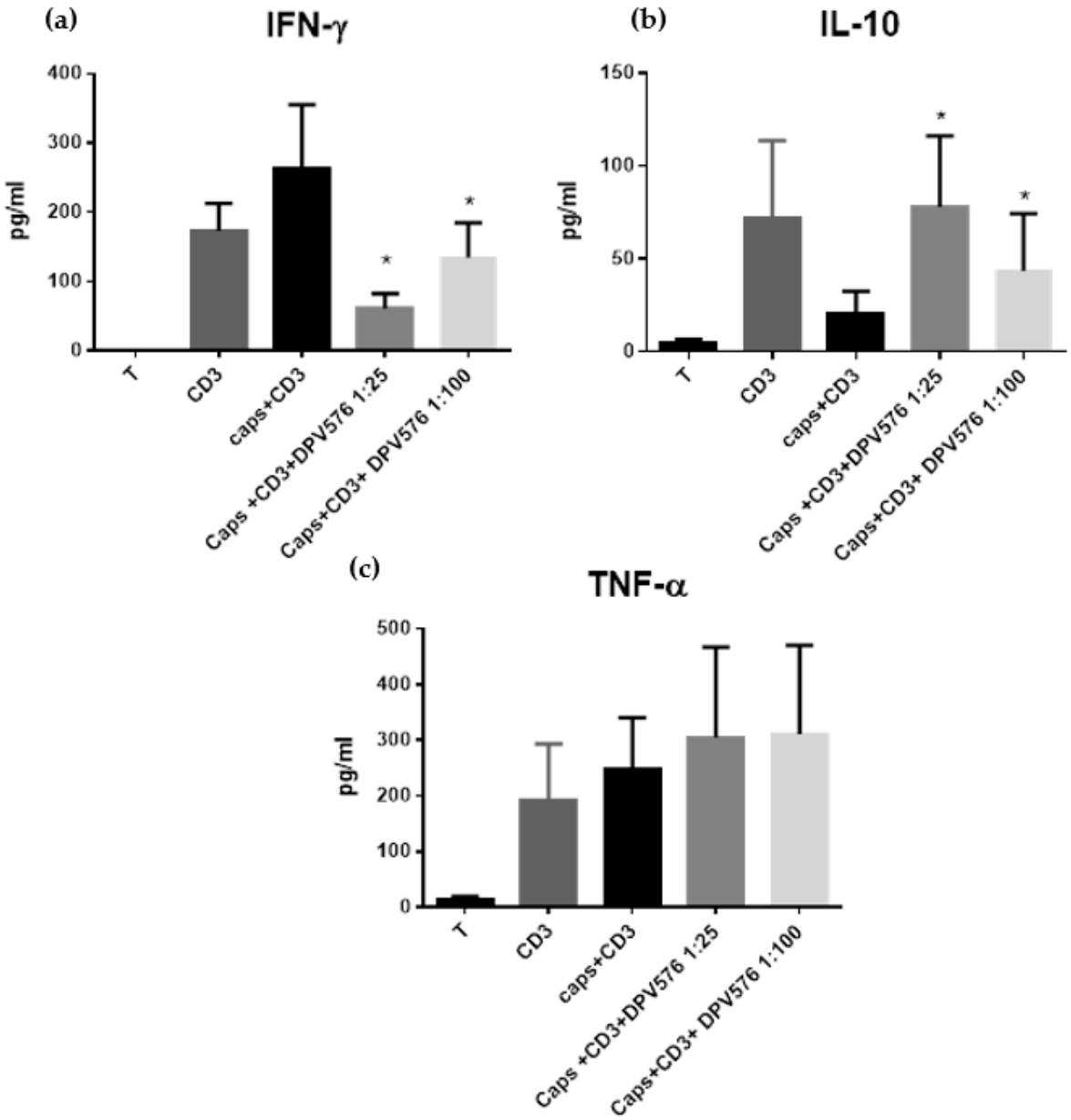

Figure 5. DPV576 modulated cytokine secretion of TRPV1 agonist capsaicin in CD4+ T cells. Capsaicin + anti-CD3/CD28-activated CD4+ T lymphocytes were exposed to DPV576 for 24 hours. Secretion of cytokines was determined by ELISA. Bar graph depicts the level of: (a) IFN-γ; (b) IL-10; (c) TNF-α. Data is mean +/- S.E. of three experiments. * $p<0.05$

4. Discussion

Several studies have demonstrated the role of TRPV1 receptors in T cell activation and functions [10,20]. CD4+ T cells also express TRPV receptors [11-12]. Here we examined whether an aqueous mixture of ND and NP, DPV576, can modulate the activity of these receptors on CD4+ T cells. We observed increased expression of TRPV1 over control cells when T cells were activated with anti-CD3 and anti-CD28 beads. Moreover, DPV576 inhibits the expression of TRPV1 in capsaicin-activated CD4+ T cells. These results show that DPV576 has the ability to modulate TRPV1 activity in CD4+ T cells, which may suggest its possible beneficial use for pain management.

Data of the current study confirm the expression of TRPV1 on CD4+ T cells. Several studies demonstrated TRPV1 mRNA and protein expression in primary mouse and human T cells [11,16,21-23] and in mouse and rat thymocytes [24-25]. Earlier studies reveal that T cell activation and release of specific inflammatory cytokines, which may be associated with immune-related diseases, are attributed to several TRP channels that are expressed in T cells [6, 26]. Special emphasis was focused

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on the role of TRPV1 that has been examined *in vivo* in models of T cell-mediated colitis, via its pro-inflammatory properties and regulation of cytokine production [11,27-28]. Data of the current study showed that short term exposure to DPV576 modulates the activity of TRPV1 channels in CD4+ T lymphocytes, which is associated with increased production of IFN-γ at both 1:25 and 1:100 dilutions of DPV576.

It has been generally accepted that TRP channel inhibitors have the ability to block the release of inflammatory mediators responsible for pain generation. For example, the discovery of archetypal thermoTRP, the vanilloid (capsaicin) receptor TRPV1, nearly two decades ago has piqued considerable interest in the scientific community [7,29-30]. TRPV1 became the focus of research into the perception of pain. Despite extensive research, the underlying mechanisms of pain are still not fully understood. In recent years, increasing evidence indicates a pivotal role of the immune system in pain [31-32]. The majority of previously published data links pain syndromes with higher levels of pro-inflammatory cytokines. Emerging evidence also suggests a role of T-lymphocytes in chronic neuropathic pain [33]. A TH1/TH2 imbalance has already been shown in patients with complex regional pain syndrome and chronic pelvic pain [34-35]. TH17 has been linked to increased pain sensitivity and destructive effects promoting persistent pain [36], while Tregs were found to be mainly involved in the endogenous recovery [37]. Our data showed the ability of DPV576 to down modulate capsaicin-induced TRPV1 and IFN-γ on CD4+ T cells, suggesting that these cells play a central role in pain management and also illustrates a possible beneficial effect of DPV576 in modulating pain and fatigue. Enhanced production of anti-inflammatory cytokine, IL-10 (Figure 5), will further aid in reducing pain. Furthermore, data showed that treatment with DPV576 inhibits the expression of TRPV1 in capsaicin-activated CD4+ T cells, in accordance with an earlier study showing that capsaicin is a specific TRPV1 channel agonist [38].

Pro-inflammatory cytokines can cause changes in behavior, including symptoms of fatigue, lethargy, muscle aches, cognitive dysfunction, and depressed mood [39-40]. Interestingly, results of a recent and robustly designed study by Raison et al. showed that all levels of fatigue are associated with increased inflammation, as indexed by elevated plasma C-reactive protein levels and white blood cell count, even after adjusting for depressive status [39]. This study further supports the notion that the symptom of fatigue, rather than a diagnosis of chronic fatigue syndrome (CFS) itself, may be what is clinically associated with inflammation. Decreased secretion of IFN-γ by CD4+ T cells in the presence of DPV576 may thus be beneficial in reducing fatigue and muscle aches. This could be one of the potential uses of clothes incorporating nanodiamond (ND) and nanoplatinum (NP).

5. Conclusions

Short term exposure to DPV576 inhibits the activity of TRPV1 channels in CD4+ T lymphocytes, which may suggest its possible beneficial use for pain management. To our knowledge, this study is the first investigation into these potential therapeutic properties of nanoparticles.

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Author Contributions: M.G. and A.A. conceived and designed the experiments; A.A. performed the experiments; M.G. and A.G. analyzed the data; H.K. contributed DPV576; A.G. performed background and primary literature search; M.G., J.G., and A.G. wrote and revised the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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