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The regulation of CD4+ T cell immune responses toward Th2 cell development by prostaglandin E2

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ABSTRACT

As an important immune mediator, PGE2 plays an important role in the immune tolerance, autoimmune diseases, immune regulation and tumor immunotolerance. PGE2 is considered to be a promising candidate for the control of the immune diseases. To further understand the immuno-modulating effects of PGE2 on CD4+ T cells, in vitro investigation was conducted in the present study. The results showed that PGE2 inhibited the proliferation of T cells in vitro in a dose-dependent manner. Gene expression profiling showed that 1716 genes were down regulated and 73 genes were up regulated with a change of 1.5 fold. Several signal transduction pathways were involved, such as TNF-α and NF-kB signaling pathway, T cell receptor signaling pathway, IL-2 signaling pathway, and MAPK pathway. The results showed that PGE2 inhibited IFN-γ, TNF-α and IL-4 production by CD4+ T cells 24 h after cell culture. A comparison between IFN-γ and IL-4 production showed that PGE2 enhanced the relative ratio of IL-4 to IFN-γ in CD4+ T cells culture, and regulated CD4+ T cells toward Th2 cell development. The results of the present study indicated that PGE2 has the potential to treat Th1-mediated inflammatory diseases by regulating CD4+ T cells toward Th2 cell immune response.

1. Introduction

Prostaglandin E2 (PGE2) is a lipid signaling intermediate produced by cyclooxygenation of arachidonic acid through the action of cyclooxygenase enzymes and PGE2 synthase enzymes. It participates in a broad range of physiological processes including blood clotting, ovulation, initiation of labor, bone metabolism, nerve growth and development, and wound healing [1]. PGE2 has long been recognized as an important mediator in the physiological processes of inflammation and anti-inflammatory reactions. In addition to its role in numerous physiological processes, PGE2 is synthesized by different cells, including major immune cells, and participates in various aspects of immune responses [2–5]. Studies in the past decades had revealed its important role in the immune tolerance, autoimmune diseases, immune regulation during graft versus host disease (GVHD) and tumor immunotolerance [6–8].

T cells are major effectors of many immune responses and play a key role in the development and progression of various immune diseases. CD4+ T cells can differentiate into Th1 or Th2 cells, with different cytokine profiles and functions [9]. Th1 cytokines promote the development of cell-mediated immune responses, and Th2 cytokines are necessary for humoral immunity. Previous studies demonstrated the effects of PGE2 on the regulation of immune activity of CD4+ T cell by inducing growth inhibition or by inducing differentiation of CD4+ CD25+ Foxp3+ regulatory T cells (Treg) [10]. Reports showed that PGE2 plays an important role for Th2 activation and that PGE2 primarily affects the induction phase of Th differentiation. Several other studies demonstrated that PGE2 production is enhanced in Th2 related diseases [1,11]. Based on these results, PGE2 was considered to be a promising candidate for the control of the immune disease.

The purpose of the present study is to more clearly address the role of PGE2 as a modulator of the immune response by examining its effects on CD4+ T cells in vitro. This study was conducted to find new targets of PGE2 by the method of gene expression profiling, and to address its immune regulatory mechanisms during the subsequent examination for the downstream products of the target gene.

2. Materials and methods

2.1. Reagents

RPMI 1640 medium was purchased from GibcoTM, Invitrogen Corp. Anti-CD3 and anti-CD28 monoclonal antibody were purchased

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from Diaclone SAS Inc. PGE2 was purchased from Cayman chemical. CD4 magnetic-activated cell sorting (MACS) kit was purchased from Miltenyi Biotec. IL-2, IL-4, IFN-γ and TNF-α ELISA kits were purchased from Jingmei Biosciences. TRIzol Reagent was purchased from Invitrogen. For the flow cytometric analysis, FITC-mouse anti-human CD4 monoclonal antibody (mAb), FITC-mouse anti-human CD40 mAb, FITC-mouse IgG1 negative control, PE-mouse anti-human CD25 mAb, PE-mouse IgG1 negative control, PerCP-mouse anti-human CD69 mAb, PerCP-mouse anti-human HLA-DR mAb and PerCP-mouse IgG1 negative control were purchased from BD Biosciences. Finally, BrdU kit was purchased from Roch Co.

2.2. Isolation of peripheral blood mononuclear cells (PBMCs) and T cells

PBMCs were isolated from heparinized blood, obtained from healthy volunteers, by using density gradient centrifugation. Briefly, buffy coats were obtained from healthy donors, and PBMCs were isolated by Ficoll density gradient centrifugation. PBMCs were incubated for 2 h at 37 °C, and nonadherent lymphocytes were removed and used as T cells in the proliferation in response to TCR ligation. The processing of the blood was performed under the same conditions.

2.3. Purification of CD4+ T cell

CD4+ T cells were purified from PBMCs using CD4 magnetic-activated cell sorting (MACS) beads according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The analysis by flow cytometry with antibodies to human CD4, the purified cell population showed positivity of CD4. The presence of CD4 in the purified cell population was confirmed by flow cytometry analysis.

2.4. Proliferation assay

CD4+ T cell proliferation assays were set up in flat-bottom 96-well plates (Falcon 3047; Becton Dickinson, Franklin Lakes, NJ) with triplicate wells for each experimental condition in the same culture as described below.

CD4+ T cells (1×10^6/mL) were stimulated with anti-CD3 monoclonal antibody (mAb; 0.2 μg/mL) and anti-CD28 mAb (0.2 μg/mL) and cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), in a humidified atmosphere at 37 °C using a 5% CO2 atmosphere, in the absence or presence of PGE2 (7–28 μmol/L) for 96 h in triplicate.

The proliferation of CD4+ T cells was monitored by measuring the incorporation of 5-bromo-deoxyuridine (BrdU; Roche Diagnostics, Mannheim, Germany) on day 4 of culture. Cells were harvested 24 h after the addition of BrdU. Cell proliferation was measured by a colorimetric immunoassay using incorporated nuclear BrdU according to the manufacturer's guidelines. After removal of the culture medium, cells were fixed to the bottom of the plates and incorporated BrdU was quantified by anti-BrdU-peroxidase antibody and tetramethylbenzidine (TMB) substrate. BrdU incorporation was assessed by absorbance at a wavelength of 450 nm using a multiwall enzyme-linked immunosorbent assay (ELISA) reader.

2.5. CD4+ T cell culture

We selected 14 μmol/L as assay concentration to detect the effect of PGE2 on the production of cytokines associated with the aforesaid signaling pathways of CD4+ T cells.

1×10^6 CD4+ T-cells (1×10^6/mL) was cultured in 24-well culture plates (Falcon 3047; Becton Dickinson, Franklin Lakes, NJ) for 24 h with and without PGE2 (14 μmol/L) in the presence of anti-CD3 mAb (0.2 μg/mL) and anti-CD28 mAb (0.2 μg/mL) in RPMI 1640 medium, supplemented with 10% FCS. The experiment was conducted in triplicate wells in a humidified atmosphere at 37 °C using a 5% CO2 atmosphere. After removal of the culture medium, cells were collected to use for RNA isolation and microarray analysis.

2.6. RNA isolation

The isolation of RNA was carried out by the TRIzol Reagent according to the manufacturer's protocol (Invitrogen). Briefly, cells were washed twice in cold PBS and centrifuged at 3000 *g* for 5 min at 4 °C between each wash, the resultant pellets were resuspended in 1000 μL of TRIzol solution. A volume of 350 μL of chloroform was added, and the solution was mixed thoroughly. Mixtures were placed on ice for 10 min followed by centrifugation at 16000 *g* for 30 min. The aqueous phase was placed in fresh tubes and mixed with equal volume of ice-cold isopropanol and placed at −20 °C overnight. The RNA precipitates were pelleted via centrifuged at 16000 *g* for 30 min. The supernatant was removed. The remaining pellets were washed with 500 μL of 75% ice-cold ethanol and centrifuged at 3000 *g* for 10 min, after which the supernatants were removed and the pellets were allowed to dry at room temperature for 5 to10 min. RNA samples were finally redissolved in 10 to 20 μL RNAse-free water.

2.7. Microarray analysis

Total RNA from each sample was purified using the method described above. Hybridization and data acquisition were performed at KangChen Bio-tech, Shanghai. After RNA was measurement on the Nanodrop ND-1000 and denaturing gel electrophoresis, the samples were amplified and labeled using the Agilent Quick Amp labeling kit and hybridized with Agilent whole genome oligo microarray in Agilent's SureHyb Hybridization Chambers. After hybridization and washing, the processed slides were scanned with the Agilent DNA microarray scanner (part number G2505B).The results extracted from Agilent Feature Extraction Software (version 9.5.3) were imported into the Agilent GeneSpring GX software (version 7.3 or later) for further analysis. The microarray data sets were normalized in GeneSpring GX using the Agilent FE Two-color scenario (mainly LOWESS normalization). Genes marked to have ("All Targets Value") were chosen for further analysis. Expressed genes were identified by fold change.

2.8. Enzyme-linked immunosorbent assays (ELISA)

Purified CD4+ T cells were activated by the addition of anti-human CD3 mAb and anti-human CD28 mAb in the presence or absence of 14 μmol/L PGE2. 24 h, 48 h and 72 h after beginning of culture, supernatants were harvested respectively for titration of IFN-γ, TNF-α, IL-4 and IL-2. It is done by the method of ELISA. Cells were collected 72 h after the beginning of culture for Flow cytometry analysis.

2.9. Flow cytometry analysis

The collected cells were washed twice with phosphate-buffered saline (PBS) plus 0.5% human serum albumin (HSA) and were incubated at 4 °C for 30 min with the following fluorochrome-conjugated mouse antihuman monoclonal antibodies at the concentrations indicated by the manufacturer's instructions: anti-CD25, anti-CD69, anti-CD40, and anti-HLA-DR (BD Biosciences, Heidelberg, Germany). The cells were then washed and resuspended in 200 μL/sample. Dead cells were excluded. Events (10000) were collected for each sample on a FACScan flow cytometer and analyzed by flow cytometry (BD Biosciences).

2.10. Statistical analysis

Comparison between paired or unpaired groups was performed using the appropriate Student's *t* test. P values below .05 were defined as statistically significant. All statistical analyses were performed using the SPSS statistical software package (SPSS 10.0 for Windows, SPSS, Chicago, IL).

3. Results

3.1. PGE2 inhibits the proliferation of CD4+ T cells

At first, we investigated the effect of PGE2 on proliferation of CD4+ T cells stimulated by anti-human CD3 mAb and anti-human CD28 mAb. The results showed that CD4+ T cells proliferated in the presence of anti-human CD3 mAb and anti-human CD28 mAb, and PGE2 inhibit the proliferation of CD4+ T cells. As shown in Fig. 1, with the increase of PGE2 concentration, the percent growth decreased significantly (P = 0.000). There was a significant positive correlation between the percent growth inhibition of CD4+ T cells and PGE2 concentrations (correlation coefficient = 0.931, P = 0.000, Fig. 2). As TCR ligation, anti-human CD3 mAb and anti-human CD28 mAb could stimulate the proliferation of CD4+ T cells. These results demonstrated that PGE2 inhibits the proliferation of CD4+ T cells in a dose-dependent manner.

3.2. PGE2 down-regulates the express of immune regulation genes in CD4+ T cell

To study the immune regulatory effects of PGE2 on the human CD4+ T cells in vitro, gene expression profiling was conducted to reveal new targets of PGE2. We performed microarray analysis on purified CD4+ T cell samples (Fig. 3) which were stimulated by anti-human CD3 mAb and CD28 mAb in the presence or absence of PGE2.

In the proliferation assay, three different concentrations from 7 to 28 μmol/L were selected in order to observe inhibition of PGE2 to the proliferation of CD4+ T cells. The results demonstrated that PGE2 inhibits the proliferation of CD4+ T cells in a dose-dependent manner. At 7 μmol/L concentration of PGE2 inhibition of PGE2 was weak. If 7 μmol/L concentration was chosen for the CD4+ T cell culture, the effect of PGE2 on the production of cytokines associated with the aforesaid signaling pathways of CD4+ T cells maybe not significant. At 28 μmol/L concentration very strong inhibitory effects

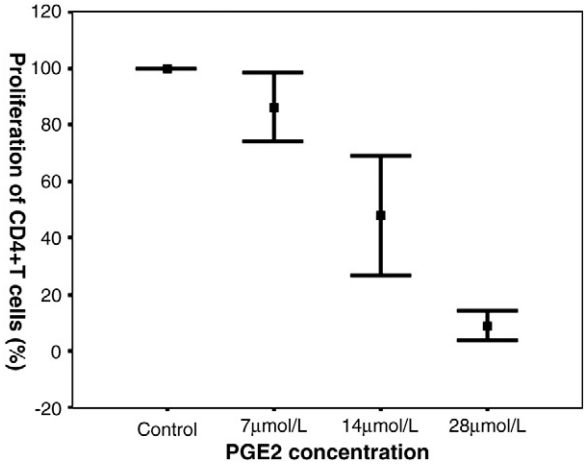


Fig. 1. Different PGE2 concentrations affect proliferation of CD4+ T cells. CD4+ T cells (1 × 106/mL) were stimulated with anti-CD3 mAb (0.2 μg/mL) in the absence or presence of PGE2 (7–28 μmol/L) for 96 h. With the increase of PGE2 concentration, the percent growth decreased significantly (P = 0.000).

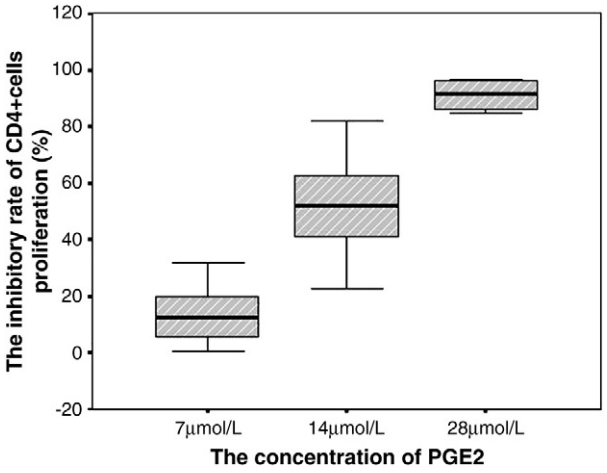


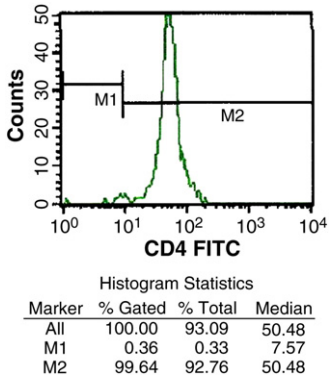
Fig. 2. There was a significant positive correlation between the percent growth inhibition of CD4+ T cells and PGE2 concentrations (correlation coefficient = 0.931, P = 0.000).

was observed, but limited cells were harvested and subsequent tests were impaired. Therefore PGE2 at 14 μmol/L concentration was chosen for the CD4+ T cell culture.

Gene expression profiling showed that, after 24 h of in vitro culture in the presence of PGE2 (14 μmol/L), 1716 genes were down regulated and 73 genes were up regulated with a fold change of 1.5. Several signal transduction pathways were involved, such as TNF-α and NF-kB signaling pathway, T cell receptor signaling pathway, IL-2 signaling pathway, MAPK pathway (Fig. 4).

3.3. The effects of PGE2 on the production of cytokines by CD4+ T cell

At concentrations from 7 to 28 μmol/L, PGE2 inhibited proliferation of T cells in a dose-dependent manner. The regulatory effect of PGE2 on CD4+ T cell secretion was tested. Four cytokines were chosen for further analysis, including TNF-α, IFN-γ, IL-2, and IL-4. As shown in Fig. 5 and Fig. 6, 24 h after the beginning of CD4+ T cell culture, PGE2 reduced the concentration of IFN-γ in the supernatant. At 24, 48 and 72 h after cell culture, IFN-γ concentrations were 0, 2.3 ± 4.7 pg/mL, and 10.9 ± 4.7 pg/mL in the test group, and 399.6 ± 332.1 pg/mL, 842.8 ± 479.7 pg/mL, and 2963.1 ± 644.9 pg/mL in the control group respectively. At several sampling points after PGE2 treatment, the concentration of IFN-γ significantly decreased, compared with the control group (P = 0.05, 0.013 and 0.001). At 24 h after cell culture TNF-α concentrations were 106.7 ± 54.5 pg/mL in the test group and 115.4 ± 58.2 pg/mL



Histogram Statistics

Marker	% Gated	% Total	Median
All	100.00	93.09	50.48
M1	0.36	0.33	7.57
M2	99.64	92.76	50.48

Fig. 3. The presence of CD4+ T cells in the purified cell population was confirmed by flow cytometry analysis. CD4+ T cells were purified from PBMCs using CD4 magnetic-activated cell sorting beads according to the manufacturer's instructions. Flow cytometry analysis of CD4+ T cells is shown (>90%).

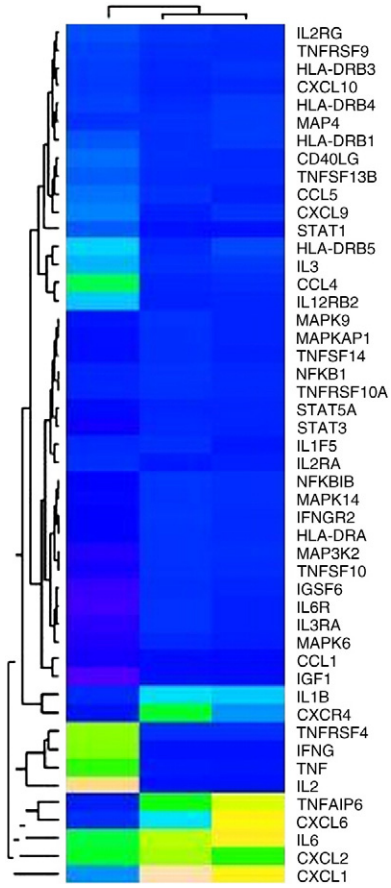


Fig. 4. PGE2 down regulates the expression of 1716 genes in CD4+ T cells. 1×106 CD4+ T cells (1×106/mL) was cultured for 24 h with PGE2 (14 μmol/L) in the presence of anti-CD3 mAb (0.2 μg/mL) and anti-CD28 mAb (0.2 μg/mL). Cells were collected for RNA isolation and microarray analysis. Data were analyzed from a comprehensive study, including TNF-α and NF-kB signaling pathway, T cell receptor signaling pathway, IL-2 signaling pathway, MAPK pathway. Shown is the heat map of the microarray results, where blue indicates down-regulation of genes and yellow indicates up-regulation of genes.

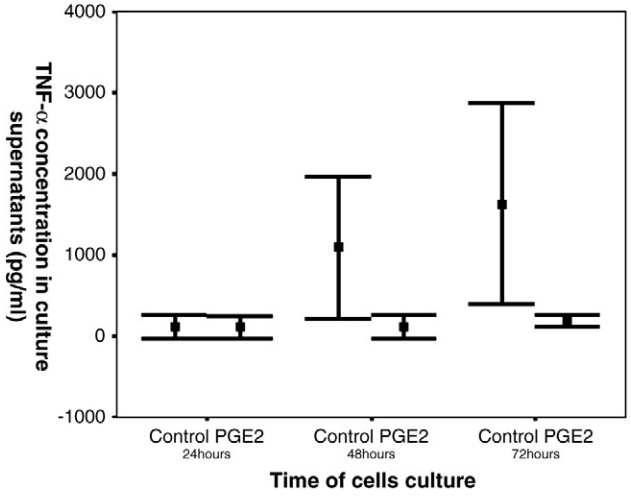


Fig. 5. PGE2 inhibited TNF-α production by CD4+ T cells 48 h and 72 h after cell culture (P = 0.009 and 0.008). PGE2 had no influence of TNF-α production by CD4+ T cells 24 h after cell culture (P = 0.188). PGE2 inhibited TNF-α production by CD4+ T cells 48 h after cell culture and the inhibitive effect is continuous for at least 72 h.

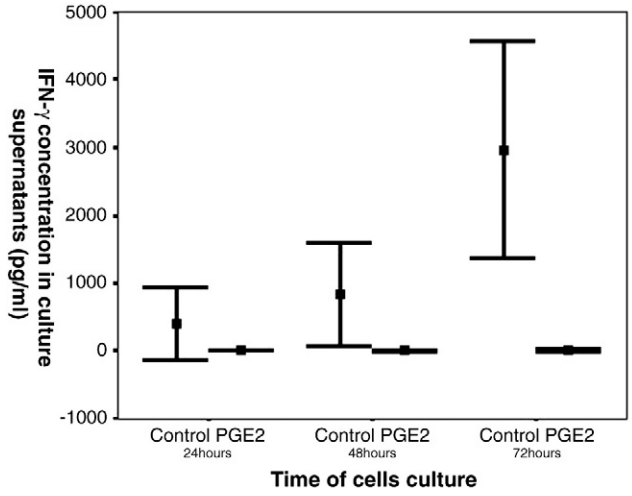


Fig. 6. PGE2 inhibited IFN-γ production by CD4+ T cells. At 24 h, 48 h and 72 h after PGE2 treatment, IFN-γ concentration significantly decreased, compared with the control group (P = 0.05, 0.013 and 0.001). The inhibitive effect of PGE2 on IFN-γ is continuous for at least 72 h.

in the control group respectively(P = 0.188). There was not a difference of TNF-α concentration at 24 h between the test group and the control group. Forty-eight hours and 72 h after cell culture, the concentrations of TNF-α were 113.5 ± 57.3 pg/mL and 189.7 ± 32.0 pg/mL in the test group, and 1093.7 ± 353.3 and 1627.2 ± 498.6 pg/mL in the control group respectively (P = 0.009 and 0.008). Therefore, 48 h after the beginning of CD4+ T cell culture, PGE2 reduced the concentration of TNF-α in the supernatant. However, the concentrations of IL-2 were below the detection threshold of ELISA kit in the treatment group as well as the control group at all sampling times. These data showed that PGE2 inhibited IFN-γ and TNF-α production by CD4+ T cells 24 h and 48 h after cell culture respectively and the inhibitive effect is continuous for at least 72 h.

The IL-4 concentrations at 24 h, 48 h and 72 h were 169.70 ± 114.90 pg/mL, 42.05 ± 19.89 pg/mL, and 27.32 ± 13.30 pg/mL respectively in control group. IL-4 had a significantly higher concentration at 24 h than that at 48 h and 72 h (P = 0.012 and 0.006) in the control group. The IL-4 concentrations at 24 h, 48 h, and 72 h were 25.41 ± 3.30 pg/mL, 21.22 ± 4.59 pg/mL, and 20.71 ± 4.77 pg/mL (P = 0.204) in the test group. The IL-4 concentrations produced did not change significantly at different sampling time in the test group. Twenty-four hours after culture, the IL-4 concentrations in the test group were significantly lower than those in control group (P = 0.048). But there was no statistical significance at 48 h and 72 h (Fig. 7).

3.4. A comparison between IFN-γ and IL-4 production

The IL-4 concentrations have an advantage in CD4+ T cells culture in the test group at 24 h. At 48 and 72 h after cell culture, the mean relative ratio of IL-4 to IFN-γ in CD4+ T cells culture was 9.23 and 1.9 in the test group, and 0.05 and 0.01 in the control group respectively. Although PGE2 inhibited IFN-γ and IL-4 production by CD4+ T cells 24, 48 and 72 h after cell culture, a comparison between IFN-γ and IL-4 production showed that PGE2 enhanced the relative ratio of IL-4 to IFN-γ in CD4+ T cells culture, and regulated CD4+ T cells toward Th2 cell development.

3.5. The effects of PGE2 on the surface molecules by CD4+ T cell

Next, we assessed the effect of PGE2 on activation markers related to the activation states of CD4+ T cells and costimulatory molecules in the process of T cells activation. As shown in Figs. 8 and 9, results of

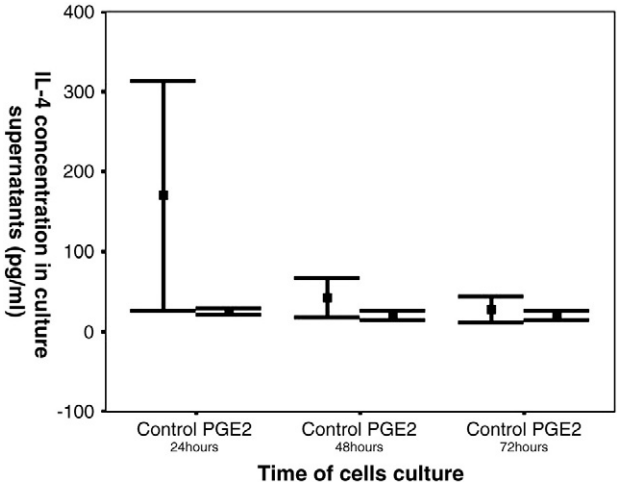


Fig. 7. PGE2 inhibited IL-4 production by CD4+ T cells at 24 h after cell culture (P = 0.048). There was no statistical significance at 48 h and 72 h after cell culture.

FCM analysis showed that the expression of CD25 CD69 HLA-DR and CD40 was reduced in the PGE2 treated group 24 h after incubation.

4. Discussion

PGE2 is generated from arachidonic acid by cyclooxygenases (COXs) and prostaglandin E synthase [1]. It is a biologically important lipid involved in processes as diverse as pain, fever and inflammation. Recent studies have shown that PGE2 is a potential candidate for the induction of tolerance in a tumor environment, for many tumor entities are described to be associated with elevated levels of PGE2 [7,8]. PGE2 was recently found to be associated with the induction of regulatory T cells [10] and T cells inhibition [11].

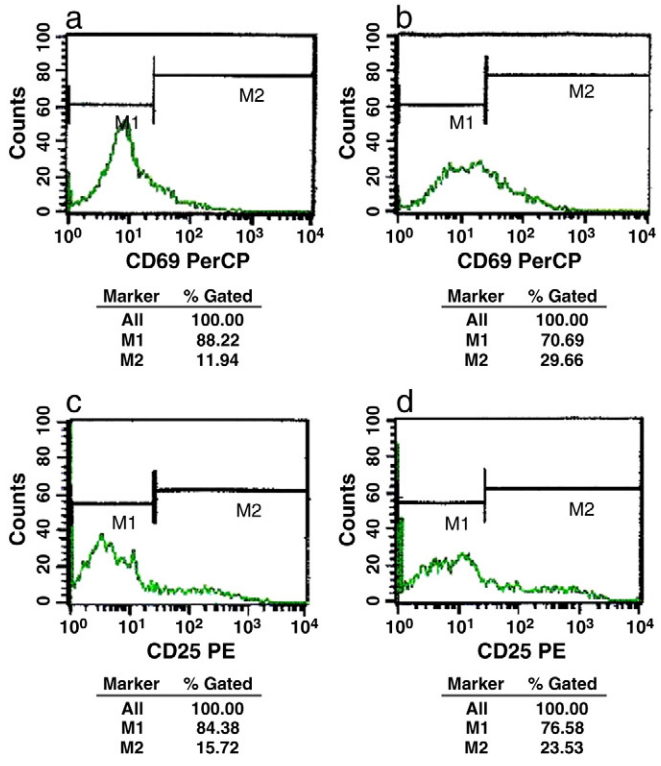


Fig. 8. The presence of activation markers in the surface of CD4+ T cells was confirmed by flow cytometry analysis. (a) The expression of CD69 in the surface of CD4+ T cells was confirmed at 24 h after cell culture without PGE2. (b) The expression of CD69 was reduced after PGE treatment. (c) The expression of CD25 in the surface of CD4+ T cells was confirmed at 24 h after cell culture without PGE2. (d) The expression of CD25 was reduced after PGE2 treatment.

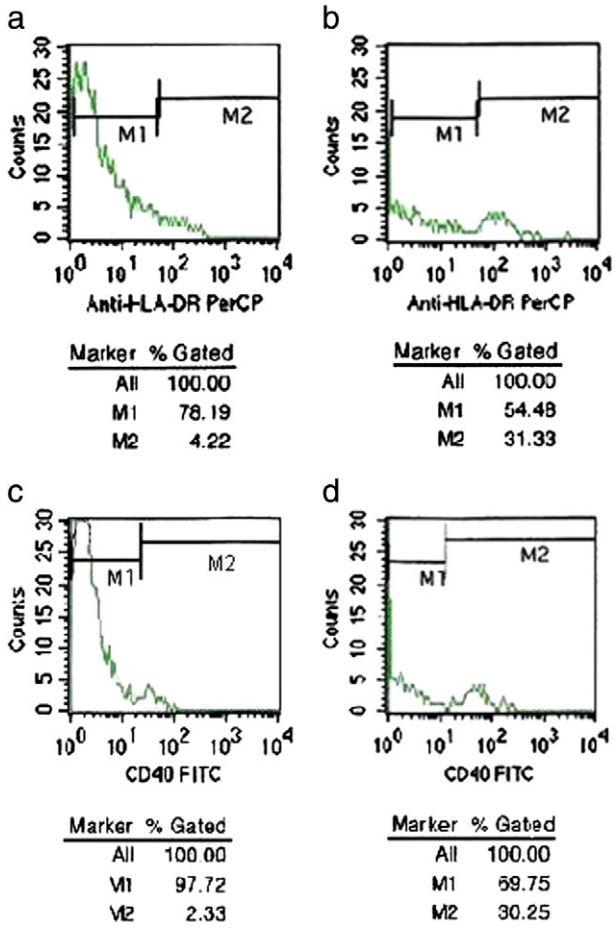


Fig. 9. The presence of costimulatory molecules in the surface of CD4+ T cells was confirmed by flow cytometry analysis. (a) The expression of HLA-DR in the surface of CD4+ T cells was confirmed at 24 h after cell culture without PGE2. (b) The expression of HLA-DR was reduced after PGE treatment. (c) The expression of CD40 in the surface of CD4+ T cells was confirmed at 24 h after cell culture without PGE2. (d) The expression of CD40 was reduced after PGE2 treatment.

The present study demonstrated that CD4+ T cells proliferated in the presence of anti-human CD3 mAb and anti-human CD28 mAb. PGE2 inhibits CD4+ T cells proliferation, and the degree of inhibition is correlated with the concentration of PGE2. With the increase concentration of PGE2, the percent growth of CD4+ T cells significantly decreased. At present, the mechanism of the inhibition is unclear. The effect of PGE2 is likely mediated by cAMP because synthetic analogs of cAMP or agents that enhance cAMP levels by modulating adenylyl cyclase or phosphodiesterase activity showed similar results. PGE2 affects both the activation and proliferation of T cells by interacting with specific cell surface receptors to stimulate cAMP accumulation, thereby activating the cAMP-dependent protein kinase A (PKA). The increased cAMP levels affect the PKA pathway by inhibiting Ca2+ mobilization and reducing the turnover of inositol3-phosphates. Recent studies demonstrated that PGE2-induced elevation of cAMP exerts a beneficial effect on allograft survival by modulating T cell function [12–14]. Additional studies have shown that cAMP elevation can alter expression and function of transcription factors involved in IL-2 and IL-2R gene expression [15]. There are also reports that activation of PKA can block DNA binding activity of NF-kB to kB sequences in the IL-2 promoter [16]. These studies illustrate that cAMP elevating agents may act at multiple levels to alter gene

expression after CD3 signaling. This immunosuppressive effect of PGE2 was demonstrated by inhibition of normal T-cell proliferation.

Previous studies have provided evidence that PGE2 modifies cytokine production of T cells and regulates immune response in vitro. The present study approached these questions by examining the effects of PGE2 on the gene expression of CD4+ T cell by microarray analysis. In this study, gene expression profiling showed that many genes associated with immune regulation, such as TNF-α and NF-kB signaling pathway, T cell receptor signaling pathway, IL-2 signaling pathway, and MAPK pathway, were down regulated in the presence of PGE2 (14 umol/L). These data revealed that PGE2 may act at multiple pathways to modulate gene expression and inhibit immune responses. In this study we analyzed cytokines associated with the aforesaid signaling pathways and explored the mechanism of PGE2 for CD4+ T cells.

CD4+ T cells have been shown to differentiate into Th1 or Th2 cells, with different cytokine profiles and functions [9]. During a normal immune response, both Th1 and Th2 cell types are involved in a cross-regulatory fashion. It is suggested that the imbalance between these subsets contributes to the development of disease: a strong Th2 response is thought to play a role in allergic diseases and antibody-mediated autoimmune diseases, whereas a dominating Th1 response might contribute to the development of cell-mediated autoimmune diseases. The differentiation of CD4+ T cells into either Th1 or Th2 cells can be influenced by several factors in the microenvironment. The presence of IL-4 during a developing immune response has been shown to favor Th2 responses. On the other hand, IFN-γ and TNF-α have been shown to be crucial factors in the development of Th1 responses. Previous studies showed that PGE2 inhibits the production of other Th1 cytokines such as IFN-γ, without affecting the production of the Th2 cytokines IL-4 [1,11]. Unlike previous studies, the study on dynamic effect of PGE2 on cytokine production by CD4+ T cells indicates that PGE2 influences production of Th1 and Th2 cytokines and suppresses the production of IFN-γ, and significantly influences the peak of IFN secretion by CD4+ T lymphocyte. The effect of PGE2 on IL-4 production by CD4+ T cells was observed at 24 h of culture, but its continuous effect was not significant. Although PGE2 down regulated IL-4 production of CD4+ T cells, a comparison between IFN-γ and IL-4 production showed that the relative suppressive effects of PGE2 were consistent with the fact that the suppressive effect of PGE2 on the IFN-γ production was higher than on the IL-4. PGE2 down regulated IFN-γ production of CD4+ T cells and modulated the differentiation of CD4+ T cells into Th2 cells. PGE2 enhances the relative ratio of IL-4 to IFN-γ in the environment of CD4+ T cells culture, and regulates CD4+ T cells toward Th2 cell development.

TNF-α is a potent pro-inflammatory cytokine with immunoregulatory activities and usually associated with Th1 responses. In the present study, TNF-α production was down regulated in 48 h after cell culture. TNF-α reduction lagged behind IFN-γ reduction. Therefore, TNF-α reduction further enhanced the role of IFN-γ inhibiting Th1 response and had an advantage of Th2 responses.

In conclusion, PGE2 favors Th2 cell development in CD4+ T cells by inhibiting the production of the Th1-associated cytokines and Th2 associated cytokines.

IL-2 plays a pivotal role in regulating the immune responses by controlling the survival and proliferation of T cells, which are required for the maintenance of immune balance. There were two postulations to explain the lack of measurable IL-2 response in both the test and control groups. First, IL-2 concentration was too low to be measured. Second, the production of IL-2 by CD4+ T cells was not regulated by PGE2.

To further study the function of IL-2 receptor and to determine the correlation between the sensitivity to PGE2 and the activation status of CD4+ T cells, we analyzed the activation markers of CD4+ T cell by the method of FCM. Among the tested markers, only CD69 and CD25 are discussed here. CD69 and CD25 are important symbols for the T cell activation [17,18]. The results of this study demonstrated that the expression of CD25 and CD69 was reduced 24 h after PGE treatment. As an IL-2 receptor, CD25 plays an important role in the immune injury mediated by IL-2. A previous study showed that blocking CD25 could effectively control some pathologic process associated with cell-mediated immune responses [15]. Because there was no IL-2 production in the culture supernatant in this study, we assumed that the immune regulation of PGE2 to IL-2 signaling pathway maybe achieved by down-regulating the CD25 expression.

HLA-DR and CD40 are important co-stimulatory molecules in the process of T cells activation. Our studies show that PGE2 can lead to reduction in surface expression of HLA-DR and CD40 after stimulation via CD3. Consistent with the result of this study, Morichika T. [19] found that PGE2 could reduce the expression of intercellular adhesion molecule and CD80/86 co-stimulatory molecules, and inhibit mixed lymphocyte reaction. There is a possibility that those surface molecules that appear to play a role in CD4+ T cells activation through the TCR were important targets of PGE2 in the immune regulation. However, the precise function of PGE2 through those molecules is not completely understood.

Based on these observations, the role of PGE2 in immune regulation of the CD4+ T cells was elucidated. Regulating effects on inflammatory mediators and surface molecules related to CD4+ T cell activation were observed. However the mechanism of PGE2 in regulating IFN-γ and TNF-α is still not fully understood. Further studies are need in this area.

In conclusion, enhanced PGE2 production reduced Th1 cytokine levels, exempting Th2 cytokines. The present study, along with other studies in the literature, provides an immunological rationale to impress Th1 immune response by PGE2. Therefore, the inhibition of Th1-mediated inflammatory diseases by stable PGE2 analogs combined with immune-based therapy is a novel concept that needs further exploration in preclinical animal models and in clinical settings.

Disclosure

All the authors declared no competing interests.

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