

***In vitro* effects of eicosanoids derived from different 20-carbon fatty acids on T helper type 1 and T helper type 2 cytokine production in human whole-blood cultures**

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Summary

Background Different series prostaglandins (PGs) and leukotrienes (LTs) are synthesized from different 20 carbon fatty acid precursors. The effects of the different series of PGs and LTs on production of T helper type 1 (Th1) and Th2 cytokines by human cells are not well established.

Objective To characterize the influence of PGs and LTs produced from different fatty acid precursors on the Th1 and Th2 cytokine profile in mitogen-stimulated human whole-blood cultures.

Methods Blood from healthy adult males was diluted and cultured with concanavalin A in the presence or absence of a range of concentrations of various PGs or LTs. Cytokine concentrations in culture supernatants were analysed by enzyme-linked immunosorbent assay (ELISA).

Results PGE₁, PGE₂ and PGE₃ significantly and dose-dependently decreased the concentrations of the Th1 cytokines IL-2 and IFN- γ by up to 50% and 70%, respectively. The three PGs exhibited similar potency towards IFN- γ production. At the highest concentration used (10⁻⁶ M) PGE₁, but not PGE₂ or PGE₃, increased the concentration of the Th2 cytokine IL-4 by about 70%. IL-10 production was not affected by PGs. The ratio of the concentrations of IFN- γ to IL-4 was significantly decreased at PGE concentrations of 10⁻⁷ and 10⁻⁶ M with all three PGEs having similar effects. LTB₄, LTC₄ and LTC₅ did not significantly affect production of the cytokines studied.

Conclusion PGE produced from different fatty acids significantly decrease Th1 cytokine production resulting in a shift in the Th1, Th2 balance in favour of a Th2 response. PGE produced from different fatty acid precursors are equipotent in their effects on human T lymphocytes. Thus, although changes in the pattern of dietary fatty intakes may contribute to the increased prevalence of atopic disease, this would probably not be mediated through substitution of one PGE with another from a different series. It may, however, be mediated through a change in the total amount of PGE produced at the site of antigen presentation.

Keywords allergy, asthma, cytokine, eicosanoid, fatty acid, interleukin, leukotriene, prostaglandin, Th1, Th2

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Introduction

There has been an increase in the prevalence of atopic disease in the last four decades, particularly in more affluent societies [1–3]. Such an increase must be due to alterations in environmental factors. The time scale of this increase is mapped by large changes in fat intake, particularly in terms of quality [4]. Over this period of time there was a decrease in the intake of saturated fats and an increase in the intake of vegetable oil-based products, rich in the n-6 family of polyunsaturated fats [4]. The major n-6 polyunsaturated fatty acid consumed in the human diet is linoleic acid, and this fatty acid is converted to arachidonic acid (Fig. 1). Human inflammatory cells contain

high proportions of arachidonic acid [5, 6] and its principal functional role is as a substrate for the synthesis of the eicosanoid family of mediators [7]. Arachidonic acid gives rise to the 2-series prostaglandins (PGs) and the 4-series leukotrienes (LTs; Fig. 1). There is a second major class of polyunsaturated fatty acids, the n-3 fatty acids. As the consumption of vegetable oil-based products has increased, the consumption of n-3 polyunsaturated fatty acids has decreased in both absolute and relative (to the n-6 family) terms [8]. Oily fish and fish oils are rich in the longer chain n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA). The pattern of change in dietary fatty acid intake over the last 40 years has been suggested to be responsible for the increase in atopic disease [9–12]. This is because eicosanoids synthesized from arachidonic acid are mediators of atopic disease [13, 14] and appear to promote the development of the T helper type 2 (Th2) phenotype that underlies atopic disease. For example, culture of human T cells with PGE₂ decreases the production of the

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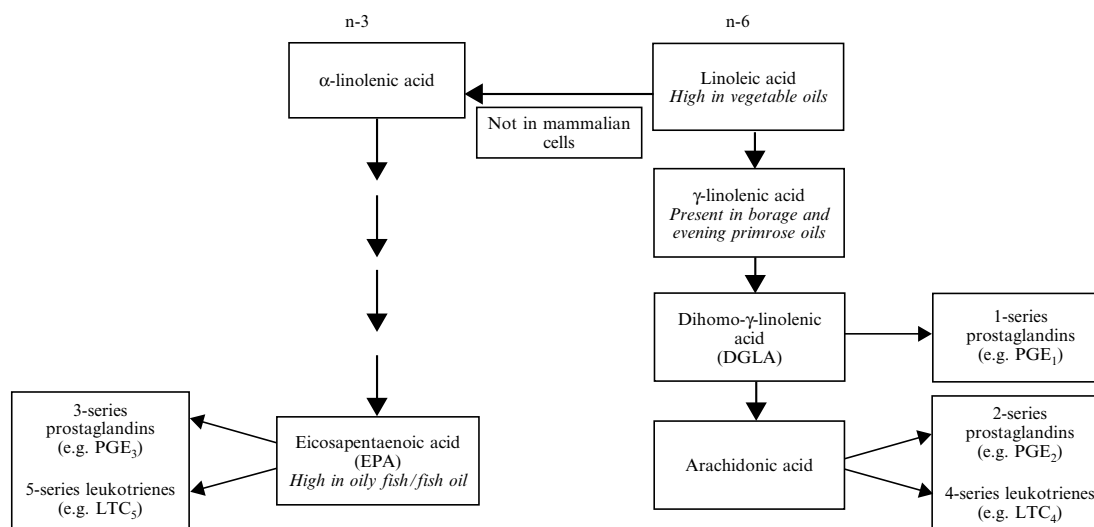


Fig. 1. An outline of polyunsaturated fatty acid metabolism. Different series of eicosanoids are synthesized from different 20 carbon polyunsaturated fatty acid precursors (DGLA, arachidonic acid and EPA). The precursors of these 20 carbon fatty acids (i.e. linoleic and α -linolenic acids) cannot be synthesized *de novo* in mammalian cells.

Th1-derived cytokines IFN- γ and IL-2 [15–18], which would be expected to relieve the Th1-mediated inhibition of development of the Th2 phenotype [19]. Studies on the effects of PGE₂ on production of Th2 cytokines, such as IL-4 and IL-10, are fewer and less conclusive with some showing increases, some decreases and others no effect on these cytokines [17, 18, 20–22]. Further evidence that PGE₂ may have a role in regulating T helper cell differentiation comes from a study in which human peripheral blood T cells cultured with stimulated monocytes produced lower levels of IFN- γ than those cultured with unstimulated monocytes [23]; monocytes are a good source of PGE₂. Furthermore, this lowered IFN- γ production could be prevented by the addition of a cyclo-oxygenase (COX) inhibitor to the stimulated monocytes, which would inhibit PGE₂ production [23]. Thus, it appears that PGE₂ suppresses Th1 cytokine production, so favouring a Th2 bias. The actions of 4-series leukotrienes on lymphocytes are less well characterized, although the LTB₄ receptor is constitutively expressed on both resting and stimulated T cells [24]. Addition of a LTB₄ antagonist to T cell cultures resulted in decreased production of IL-2, IFN- γ and IL-4 [24], suggesting that LTB₄ enhances production of both Th1 and Th2 cytokines, while LTB₄ induced a concentration-dependent increase in IL-5 production by T cells [25].

Because arachidonic acid-derived eicosanoids are potent mediators of atopic inflammation and because PGE₂ appears to promote the development of the Th2 phenotype, it has been suggested that dietary strategies can be used to prevent and to treat atopic disease (see [26] for references). One suggestion has been to increase the intake of n-3 fatty acids from fish oil [9–12]. This is because when fish oil is consumed the amount of arachidonic acid in inflammatory cells is decreased [5], so decreasing the production of arachidonic acid-derived eicosanoids such as PGE₂ [27–29] and LTB₄ [5, 6]. When fish oil is provided in the diet, arachidonic acid is partly replaced in inflammatory cell membranes by the n-3 fatty acid EPA [5]. EPA is also a precursor for eicosanoids, giving rise to 3-series PGs and 5-series LTs [e.g. 5, 30] (Fig. 1). Although LTB₅ is a much less potent chemotactic agent than LTB₄ [31], we are not aware of any published

studies comparing the effects of eicosanoids derived from arachidonic acid and EPA on production of Th1 and Th2 cytokines.

One other 20-carbon fatty acid, dihomog- γ -linolenic acid (DGLA) is also an eicosanoid precursor, giving rise to 1-series PGs (Fig. 1). DGLA accumulates in inflammatory cell membranes when its precursor, γ -linolenic acid which is found in evening primrose and borage oils, is consumed. Consumption of these oils is also a suggested strategy in atopic disease (see [26] for references).

The relative effects of eicosanoids produced from the different 20-carbon fatty acid precursors on cytokine production by T cells have not been documented. Therefore, in this study we examined the effects of PGs and LTs of different series on the production of Th1 and Th2 cytokines by cultured human whole blood.

Materials and methods

Subjects

Blood was taken into heparin from healthy male volunteers ($n = 10$), aged 18–25 years, who had fasted for 12 h.

Chemicals

HEPES-buffered RPMI medium was purchased from Autogen Bioclear (Calne, UK). Glutamine, antibiotics (penicillin and streptomycin), concanavalin A (Con A), LTC₄ and LTC₅ were purchased from Sigma Chemical Co (Poole, UK). PGE₁, PGE₂, PGE₃ and LTB₄ were manufactured by Cayman Chemical Co. and supplied by Alexis Corp. Ltd (Nottingham, UK). Enzyme-linked immunosorbent assay (ELISA) kits for measuring cytokine concentrations were purchased from Biosource Europe (Nivelles, Belgium). ELISA kits for measuring PGE₁ concentrations were from R & D Systems Europe (Abingdon, UK). ELISA kits for measuring PGE₂ concentrations were from Neogen Corp. (Lexington, KY, USA).

Cytokine production by whole-blood cultures – effect of addition of eicosanoids

Heparinized blood was diluted 1 in 5 with RPMI-1640 medium supplemented with 0.75 mM L-glutamine and antibiotics (50 µg/mL each of benzyl penicillin and streptomycin sulphate). The diluted blood (1.7 mL/well) was placed in the wells of a 24-well culture plate. Eicosanoids were added to give final concentrations of 10^{-9} , 10^{-8} , 10^{-7} and 10^{-6} M for the PGs, and 10^{-11} , 10^{-10} , 10^{-9} , 10^{-8} and 10^{-7} M for the LTs. Eicosanoids were added in 100 µL of 2% ethanol to give a final concentration of 0.1% ethanol in the culture. Control cultures contained 0.1% ethanol but no eicosanoid. Con A (0.2 mL; 50 µg/mL final concentration) was added to stimulate cytokine production by T lymphocytes. Cultures were incubated at 37 °C in a 5% CO₂ atmosphere for 48 h. Preliminary studies indicated that 48 h was the time at which maximal concentrations of IFN-γ, IL-4 and IL-10 occurred in this culture system. At the end of the culture period the plates were centrifuged at 300 g for 5 min. The supernatant medium was then carefully removed to avoid disturbance and aliquots were frozen at –20 °C until analysis.

Cytokine production by whole-blood cultures – effect of time of addition of PGs

Diluted whole-blood cultures were set up in 24-well plates as described above. Prostaglandins were added, to give a final concentration of 10^{-6} M in 0.1% ethanol, 4 h before stimulation, at the same time as the stimulant, or 4 h after stimulation. The stimulant was Con A (50 µg/mL final concentration). Control cultures contained 0.1% ethanol but no eicosanoid. Cultures were incubated and processed as described above.

Cytokine measurements

Cytokine (IFN-γ, IL-2, IL-4, IL-10) concentrations in the culture supernatants were measured by ELISA in accordance with the manufacturer's instructions. Limits of detection for these assays were 2 pg/mL (IL-4), 1 pg/mL (IL-10), 0.1 U/mL (IL-2) and 0.03 IU/mL (IFN-γ).

PGE₁ and E₂ production by whole-blood cultures

Diluted whole-blood cultures were set up in 24-well plates and cultured as described above, except that exogenous eicosanoids were not added; cultures contained 0.1% ethanol. PGE₁ and PGE₂ concentrations in the culture supernatants were measured by ELISA in accordance with the manufacturers' instructions. Lower limits of detection were 1.7×10^{-11} M for the PGE₁ kit and 2.8×10^{-10} M for the PGE₂ kit. Cross-reactivity of the PGE₁ assay with PGE₂ is 6.5% and with PGE₃ 2.2%. Cross-reactivity of the PGE₂ assay with PGE₁ is 100% and with PGE₃ 17.7%.

Statistical analysis

Cytokine concentrations are presented as mean ± SEM. The effects of PG or LT subtype and concentration were determined by two-factor ANOVA. Where the effect of concentration was significant, one-factor ANOVA was used to determine differences among concentrations of a particular eicosanoid. These analyses included the no-eicosanoid added controls. The effects of PG subtype and time of PG addition to the cultures were determined by two-factor ANOVA. Where the effect of PG

subtype was significant, one-factor ANOVA was used to compare the effects of PGs with one another. Where the effect of time of addition was significant, one-factor ANOVA was used to determine differences among times of addition of a particular PG. All analyses were performed using SPSS version 10 for Windows (SPSS Inc., Chicago, IL, USA). A value for $P < 0.05$ was taken to indicate a statistically significant difference.

Results

PGE production by whole-blood cultures

The concentration of PGE in Con A-stimulated whole-blood cultures, measured using the PGE₂ assay, was always less than 10^{-9} M with a mean value of 6×10^{-10} M. As this assay exhibits 100% cross-reactivity with PGE₁, this concentration represented the sum of PGE₁ plus PGE₂. The mean concentration of PGE₁, measured using the PGE₁ assay which has only weak cross-reactivity with PGE₂, was 4×10^{-11} M. Thus, the contribution of PGE₂ to total PGE measured was > 90%.

Effects of addition of PGs on Th1 and Th2 cytokine production by human whole-blood cultures

IL-2 The concentration of IL-2 in the absence of added eicosanoids was 6.6 ± 0.9 U/mL. There was a significant effect of PGE concentration ($P < 0.001$) but not subtype on IL-2 concentration. All three PGs caused a dose-dependent decrease in IL-2 concentration (Table 1). PGE₁ and PGE₂ were significantly inhibitory at concentrations of 10^{-8} , 10^{-7} and 10^{-6} M, with a maximum inhibition of 35% and 45%, respectively (Table 1). The potency of these two eicosanoids was similar, with a 25% decrease in IL-2 concentration occurring at a PGE concentration of about 10^{-9} M. PGE₃ also decreased IL-2 concentration, but appeared to be less potent than the other two PGE tested. IL-2 concentration was not significantly decreased until a PGE₃ concentration of 10^{-6} M was used (Table 1) and a PGE₃ concentration of about 10^{-7} M was required to cause a 25% decrease in IL-2 concentration. Nevertheless, all three PGE caused a similar degree of inhibition of IL-2 production when used at a concentration of 10^{-6} M (Table 1).

IFN-γ The concentration of IFN-γ in the absence of added eicosanoids was 233 ± 29 IU/mL. There was a significant effect of PGE concentration ($P < 0.001$) but not subtype on IFN-γ

Table 1. Effect of exogenous prostaglandins on the concentration of IL-2 in human whole-blood cultures

PGE concentration	IL-2 concentration (U/mL)			
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M
PGE ₁	4.9 ± 0.9	$4.4 \pm 0.8^*$	$3.9 \pm 0.6^*$	$3.8 \pm 0.5^*$
PGE ₂	4.8 ± 1.3	$4.5 \pm 1.0^*$	$3.8 \pm 0.6^*$	$3.5 \pm 0.8^*$
PGE ₃	5.8 ± 1.3	5.4 ± 1.3	5.1 ± 1.3	$3.2 \pm 0.6^*$

Human diluted whole blood cultures ($n = 4-10$) were stimulated for 48 h with 50 µg/mL concanavalin A in the presence of prostaglandins at final concentrations of 10^{-9} M ($n = 4$), 10^{-8} M ($n = 4$), 10^{-7} M ($n = 4$) and 10^{-6} M ($n = 10$). IL-2 concentrations measured by ELISA are presented as mean ± SEM. The concentration of IL-2 in the absence of added eicosanoids was 6.6 ± 0.9 U/mL ($n = 10$). *Significantly different from control cultures (i.e. no added eicosanoid).

concentration. All three PGs caused a dose-dependent decrease in IFN- γ concentration (Table 2). They were each significantly inhibitory at concentrations of 10^{-8} M and above, with a maximum inhibition of 65–70% (Table 2). The potency of the PGs was similar with a 50% decrease in IFN- γ concentration occurring at a PGE concentration of about 10^{-8} M (see Fig. 3 below).

IL-4 The concentration of IL-4 in the absence of added eicosanoids was 14.6 ± 1.8 pg/mL. There was a significant effect of PGE concentration ($P=0.003$) but not subtype on IL-4 concentration. A bi-phasic pattern of effect of PGs on IL-4 concentration was seen (Table 3). At the lower PGE concentrations a decrease in IL-4 concentration was seen. This was significant for PGE₁ at a concentration of 10^{-9} M and for PGE₃ at concentrations of 10^{-9} and 10^{-8} M (Table 3). At the latter concentration PGE₃ decreased IL-4 concentration by 40%. PGE₂ at these concentrations did not have a significant effect on IL-4 concentration (Table 3). In contrast to the effects observed when PGE were used at low concentrations, at the higher concentrations they tended to increase IL-4 concentration and this effect was significant for PGE₁ (Table 3).

IL-10 The concentration of IL-10 in the absence of added eicosanoids was 342 ± 68 pg/mL. Neither PGE concentration nor subtype significantly affected IL-10 concentration (data not shown).

Table 2. Effect of exogenous prostaglandins on the concentration of IFN- γ in human whole-blood cultures

PGE concentration	IFN- γ concentration (IU/mL)			
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M
PGE ₁	192 ± 55	$138 \pm 47^*$	$100 \pm 29^*$	$65 \pm 18^*$
PGE ₂	220 ± 71	$119 \pm 16^*$	$90 \pm 41^*$	$71 \pm 19^*$
PGE ₃	218 ± 55	$121 \pm 21^*$	$92 \pm 27^*$	$84 \pm 25^*$

Human diluted whole blood cultures ($n=4-10$) were stimulated for 48 h with 50 μ g/mL concanavalin A in the presence of prostaglandins at final concentrations of 10^{-9} M ($n=4$), 10^{-8} M ($n=4$), 10^{-7} M ($n=4$) and 10^{-6} M ($n=10$). IFN- γ concentrations measured by ELISA are presented as mean $\pm$ SEM. The concentration of IFN- γ in the absence of added eicosanoids was 233 ± 29 IU/mL ($n=10$). *Significantly different from control cultures (i.e. no added eicosanoid).

Table 3. Effect of exogenous prostaglandins on the concentration of IL-4 in human whole-blood cultures

PGE concentration	IL-4 concentration (pg/mL)			
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M
PGE ₁	$9.5 \pm 1.6^*$	13.1 ± 3.6	24.3 ± 4.3	$24.4 \pm 4.0^*$
PGE ₂	12.9 ± 1.4	14.2 ± 1.4	17.2 ± 3.4	19.6 ± 3.6
PGE ₃	$10.5 \pm 1.0^*$	$8.9 \pm 2.6^*$	15.2 ± 4.5	18.7 ± 3.1

Human diluted whole blood cultures ($n=4-10$) were stimulated for 48 h with 50 μ g/mL concanavalin A in the presence of prostaglandins at final concentrations of 10^{-9} M ($n=4$), 10^{-8} M ($n=4$), 10^{-7} M ($n=4$) and 10^{-6} M ($n=10$). IL-4 concentrations measured by ELISA are presented as mean $\pm$ SEM. The concentration of IL-4 in the absence of added eicosanoids was 14.6 ± 1.8 pg/mL ($n=10$). *Significantly different from control cultures (i.e. no added eicosanoid).

Th1 to Th2 cytokine ratio The ratio of Th1 to Th2 cytokines in the cultures was calculated as the ratio of IFN- γ to IL-4. There was a significant effect of PGE concentration ($P < 0.001$) but not subtype on the Th1 to Th2 ratio (Fig. 2). PGE decreased the Th1 to Th2 cytokine ratio in a dose-dependent fashion, from about 18 in the control cultures to about 5–7.5 at PGE concentrations of 10^{-7} and 10^{-6} M (Fig. 2). The pattern of effect of PGE₁ and PGE₂ was very similar. These two PGE caused a significant decrease in the Th1 to Th2 ratio at concentrations of 10^{-8} , 10^{-7} and 10^{-6} M compared to control cultures (Fig. 2). Furthermore, at concentrations of 10^{-7} and 10^{-6} M, PGE₁ and PGE₂ decreased the Th1 to Th2 ratio compared with the cultures with 10^{-9} M exogenous PGE (Fig. 2). At concentrations of 10^{-7} and 10^{-6} M, PGE₃ also significantly decreased the Th1 to Th2 ratio compared with the control and 10^{-9} M PGE₃-added cultures (Fig. 2). However, PGE₃ did not significantly decrease the Th1 to Th2 ratio at a concentration of 10^{-8} M, and the ratio at 10^{-7} and 10^{-6} M PGE₃ was different from that at 10^{-8} M (Fig. 2).

Effects of addition of LTs on Th1 and Th2 cytokine production by human whole-blood cultures

There were no significant effects of leukotriene subtype or concentration on the concentration of IL-2, IFN- γ , IL-4 or IL-10 (data not shown).

Effect of timing of addition of PGs to cultures on IL-2 and IFN- γ concentrations

PGE at a concentration of 10^{-6} M were added to cultures prior to, at the same time as, or after the Con A stimulus. The ability of PGE₁, PGE₂ or PGE₃ at a concentration of 10^{-6} M to significantly decrease IL-2 and IFN- γ concentration was confirmed (Fig. 3). As observed in the earlier experiments, the effect on IFN- γ was more potent than that on IL-2.

There was a significant effect of PG subtype ($P < 0.001$) but not of time of PG addition to the cultures on the concentration of IL-2. The effect of PG subtype was due to the difference between cultures with exogenous PGE added and those without (Fig. 3a). IL-2 concentration was decreased to the same extent

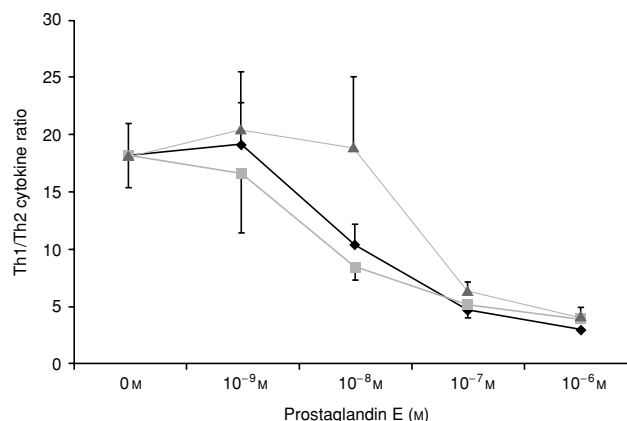


Fig. 2. Effect of exogenous prostaglandins on the ratio of Th1 to Th2 cytokines in human whole-blood cultures. Human diluted whole-blood cultures ($n=4-10$) were stimulated for 48 h with 50 μ g/mL concanavalin A in the presence of prostaglandins ($\blacklozenge$, PGE₁; $\blacksquare$, PGE₂; $\blacktriangle$, PGE₃) at final concentrations of 10^{-9} M ($n=4$), 10^{-8} M ($n=4$), 10^{-7} M ($n=4$) and 10^{-6} M ($n=10$). The ratio of IFN- γ to IL-4 (IU/pg) is used to indicate the Th1 to Th2 cytokine balance. Data are mean with SEM indicated.

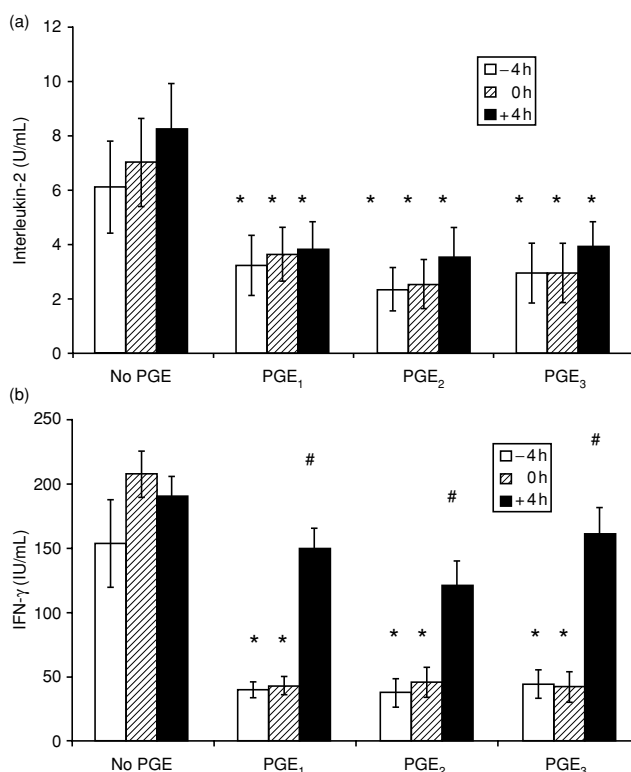


Fig. 3. Effect of timing of addition prostaglandins on IL-2 and IFN- γ concentrations in human whole-blood cultures. Human diluted whole-blood cultures ($n = 6$) were stimulated for 48 h with 50 $\mu\text{g/mL}$ concanavalin A in the presence of PGs at a final concentration of 10^{-6} M. The PGEs were added 4 h before (–4 h), at the same time as (0 h), or 4 h after (+4 h) concanavalin A. (a) IL-2, (b) IFN- γ . *Significantly different from the control (i.e. no PGE added) cultures; #Significantly different from the –4 h and the 0 h cultures (for the same PGE).

by PGE₁, PGE₂ and PGE₃, irrespective of the time of their addition to the cultures (Fig. 3a).

There were significant effects of PG subtype and time of PG addition to the cultures on the concentration of IFN- γ ($P < 0.001$ in both cases and $P = 0.005$ for subtype–time interaction). IFN- γ concentration was significantly decreased if PGs were added prior to or at the same time as the Con A stimulus (Fig. 3b). In contrast, the inhibitory effect of PGE on IFN- γ concentration did not occur if the PGE were added 4 h after the Con A stimulus (Fig. 3b).

Discussion

Lymphocytes express low activity of COX and are poor producers of COX metabolites [32, 33]. Thus, the concentration of PGs produced in cultures stimulated with T cell mitogens such as Con A is relatively low. The concentration of PGE₂ reported here for human whole-blood cultures stimulated for 48 h with Con A ($< 10^{-9}$ M) is similar to that reported previously for rat lymphocytes cultured under similar conditions (1.2×10^{-9} M [34]). In contrast, monocytes and macrophages express high activity of COX when activated and produce relatively high concentrations of PGE₂. For example, whole-blood cultures similar to those used here, but stimulated with bacterial lipopolysaccharide produced a PGE₂ concentration of

1.5×10^{-7} M [35]. Therefore, the concentrations of PGs used in this study are in excess of the concentrations produced endogenously in these cultures, but represent concentrations that would be produced by other cell types in the culture in the presence of an appropriate stimulus (e.g. lipopolysaccharide). A mean PGE₂ concentration of approximately 7.2×10^{-9} M was reported for human amniotic fluid at 16–17 weeks' gestation [36]. PGE₂ concentrations as high as 10^{-6} M can be present locally or systemically during inflammatory responses or critical illness [37]. The concentration of LTB₄ in bronchoalveolar lavage fluid from asthmatic subjects increased to 4.6×10^{-9} M post-methacholine challenge [38]. In subjects with allergic rhinitis, the concentration of LTB₄ in nasal washings increased from about 10^{-10} M to about 2×10^{-9} M after allergen challenge [39]. Thus, the concentrations of the arachidonic acid-derived PGs and LTs used here cover the physiological and pathophysiological range.

PGE₁, PGE₂ and PGE₃ had significant effects on mitogen-stimulated cytokine production by cultured human whole blood. All three PGs inhibited IL-2 and IFN- γ production, with a greater effect on the latter. There is an extensive literature reporting inhibition of IL-2 production by PGE₂ [15–18, 20, 22, 23, 40–46]. However, several of these studies used T cell clones rather than primary cultures [e.g. 16–18, 20], while others use only high concentrations of PGE₂. Furthermore, none of the earlier studies investigated the effect of PGE₂ in a whole-blood system, which more closely mimics the *in vivo* situation than cultures of purified cells [47]. Nevertheless, the results showing PGE₂-induced suppression of IL-2 production in human whole-blood cultures confirm the earlier studies in other culture settings. The observations of PGE₂-induced suppression of IFN- γ production also confirm earlier studies in other culture settings [15–18, 22, 23, 44]. However, there are very few studies that have investigated the effects of PGs of other series upon Th1 cytokine production, or that have made comparison between PGs of different series. Rappaport and Dodge [42] and Santoli and Zurier [48] showed that PGE₁ and PGE₂ equipotently inhibited IL-2 production by human peripheral blood mononuclear cells, while Gold et al. [20] showed similar effects of PGE₂ and the PGE₁ analogue misoprostol on IL-2 production by T cell clones. The current study confirms that PGE₁ inhibits production of IL-2 by mitogen-stimulated human mononuclear cells with a similar potency to PGE₂. To our knowledge there are no published studies of the effect of PGE₁ on IFN- γ production, or of the effect of PGE₃ on Th1 cytokine production by human cells. The current study indicates that PGE₁ and PGE₃ are equipotent with PGE₂ with respect to inhibition of production of IFN- γ , but that PGE₃ is less potent than the either PGE₁ or PGE₂ with respect to IL-2 production. These relative potencies towards IL-2 production can be compared with reported effects of the PGs on lymphocyte proliferation, which is largely driven by IL-2 production [49]. Santoli and Zurier [48] report similar inhibitory potency of PGE₁ and PGE₂ towards human T lymphocyte proliferation, in agreement with the similar effects of these PGs on IL-2 production observed here and elsewhere. PGE₃ was a significantly less potent inhibitor of mitogen-stimulated proliferation of rat [34] and murine lymphocytes [50], which is consistent with the lower potency of this PG towards IL-2 production by human whole-blood cultures observed in the current study. However, in contrast, PGE₃ was a more potent inhibitor of human T lymphocyte

proliferation than PGE₂ [50, 51] or PGE₁ [51]. There may be differences in sensitivity to PGE₃ between human and rodent cells, but the differences between the effects of PGE₃ on IL-2 production and proliferation by human cells suggest that PGE₃ may inhibit proliferation *in vitro* by mechanisms additional to its effects on IL-2 production. Such mechanisms might include regulation of expression of the IL-2 and transferrin receptors.

There was no effect of time of addition of PGs, relative to that of Con A, on inhibition of IL-2 production. In contrast, IFN- γ production was inhibited only if the PGs were added before or at the same time as Con A. This suggests that the mechanism of the inhibitory effect of PGs on IL-2 and IFN- γ production may be different. PGE₂, and presumably the other PGs, appears to inhibit IL-2 production through inducing high intracellular levels of cyclic AMP [45, 46] which are known to inhibit transcription of the IL-2 gene [17, 46]. The current study indicates that, if PGs do inhibit IL-2 production by this mechanism, then the elevation in cyclic AMP concentrations can be generated up to several hours post-mitogenic stimulation. The observation in the current study that IFN- γ production was not inhibited if PGs were added 4 h after mitogenic stimulation is in keeping with results from Hilkens et al. [16, 23] who found that PGE₂ gradually lost the ability to inhibit IFN- γ production if added post-stimulation, and that this effect was entirely lost by 8 h post-stimulation. The difference in the effect of timing of addition of PGs on IL-2 and IFN- γ production suggests that the latter is most likely mediated through a different mechanism from the former. Recent experiments have shown that the inhibition of IFN- γ production by PGE₂ is by maintenance of full methylation of the promoter region of the IFN- γ gene [52], consistent with the methylation pattern seen in naïve CD4⁺ T cells, which are naturally low IFN- γ producers [53–56].

Compared with the number of studies of PGs on production of Th1 cytokines, there are few studies of Th2 cytokines. Those studies that have been performed focus largely on PGE₂ and do not produce a consistent picture of its effect. PGE₂ has been reported to increase [22], decrease [21] or have no significant effect on [17, 18] IL-4 production. In a study of human T cell clones, PGE₂ (and the PGE₁ analogue, misoprostol) did not effect IL-4 production [19]. Exogenous PGE₂ had no effect on IL-4 production by human Th2 clones but at certain concentrations decreased IL-4 production by human peripheral blood lymphocytes [15]. PGE₂ increased production of the Th2 cytokine IL-5 by human peripheral blood lymphocytes [15–17] and increased IL-10 mRNA levels in cultured human T cells [57]. Taken together these indicate that, at least under certain culture conditions, PGE₂ can enhance the production of Th2 cytokines by human T cells. Under the conditions used in the current study (mitogen-stimulated diluted whole blood), IL-4 concentration was decreased by the addition of PGE₁ and PGE₃ at the lower concentrations and enhanced by all three PGs at the higher concentrations. This bi-phasic pattern of effect of the PGs on IL-4 production may in part explain some of the discrepancies in the existing literature. The mechanism by which PGs influence production of IL-4 is not clear. IL-4 production does not appear to be regulated by intracellular cyclic AMP concentrations [17, 58]. However, the promoter region of the IL-4 gene is subject to methylation that affects its transcription and the pattern of methylation appears to be the opposite of that of the IFN- γ gene promoter region [59]. Thus, a mechanism by which PGs promote hypermethylation of the IFN- γ gene

promoter region so inhibiting IFN- γ production, may also act to promote hypermethylation of the IL-4 gene so enhancing IL-4 production.

Overall, the results of the current study show that PGE derived from DGLA, arachidonic acid or EPA (i.e. PGE₁, PGE₂ or PGE₃) can inhibit the production of Th1 cytokines by human lymphocytes. PGE at a high concentrations may also enhance production of the Th2 cytokine IL-4. The calculated ratio of IFN- γ to IL-4 used as a surrogate for the ratio of Th1 to Th2 activity indicated that all three PGE studied produce a significant dose-dependent bias towards the Th2 phenotype. If these findings are to be relevant to the process of sensitization to allergens, which may occur early in life [see 36], then the concentration range over which PGs exert their effects must relate to that present at the time of sensitization. Jones et al. [36] report a range of PGE₂ concentrations in human amniotic fluid collected at 16–17 weeks of gestation of about 4×10^{-9} M to about 1.3×10^{-8} M. The results of the current study suggest that variations in PGE₂ concentration over this range will influence the generation of Th1 cytokines. However, the current study investigated the effect of exogenously added PGs on cytokine generation by cells from healthy adult males, rather than by cells predisposed to atopic development and it will be important to confirm the effects of PGs in the latter setting.

Exogenous LTB₄, LTC₄ or LTC₅ at concentrations up to 10^{-7} M had no significant effect on the concentrations of any of the cytokines studied. This contrasts with the limited number of studies that suggest that LTs affect cytokine production by T cells. Co-culture of murine alveolar macrophages with murine T cells resulted in increased IL-2 concentration, which could be attenuated by the addition of an inhibitor of LT synthesis [60]. In another report, it was observed that the LTB₄ receptor was constitutively expressed on resting and activated murine T cells and that the addition of an antagonist to this receptor resulted in reduced IL-2, IFN- γ and IL-4 production [24]. LTB₄ increased IL-5 production by murine T cells in a concentration-dependent fashion [25]. The differences between these studies and the current study may be due to different cell types investigated (the previous studies all used murine T cells) or to differences in the culture systems.

The aim of the current study was to investigate the effect of eicosanoids on production of Th1 and Th2 cytokines by human cells. The eicosanoids used were derived from different fatty acid precursors from the n-3 and n-6 families [Fig. 1]. The LTs were without effect; the overall effect of the three PGs was similar. The increased dietary intake of n-6 polyunsaturated fatty acids and the decreased intake of n-3 polyunsaturated fatty acids over the last four decades has been suggested as a possible cause of the concurrent increase in the prevalence of atopic disease [9–12]. Allergic disease is characterized by a bias in favour of Th2 cytokine production [61], and thus a factor which favours this bias may predispose individuals to develop atopic responses. This has led to a proposed speculative mechanism by which increased n-6 fatty acid intake predisposes to, and increased n-3 fatty acid intake protects against, atopic disease. The underlying assumption is that the eicosanoids produced from EPA are less potent in influencing T cell activity than those produced from arachidonic acid. The results of the current study show that PGE₂ (derived from arachidonic acid) and PGE₃ (derived from EPA) are equipotent in their ability to down-regulate the Th1 cytokine IFN- γ and have an equivalent

enhancing effect on IL-4 production at high concentrations. Thus, changes in the potency of eicosanoids produced from arachidonic acid compared with other precursors, particularly EPA, appear unlikely to explain the altered disease incidence. To our knowledge, this is the first study to generate data to examine this assumption and to enable this conclusion to be reached. However, the current study has examined the effect of exogenous addition of equivalent concentrations of eicosanoids derived from the different precursors. It has not examined the change in production of the eicosanoids as a result of different dietary regimens. When fish oil, containing EPA, is consumed in the diet, EPA is incorporated into inflammatory cell membrane phospholipids partly at the expense of arachidonic acid [5, 29, 62–64], so decreasing the substrate available for synthesis of 2-series PGs, resulting in decreased PGE₂ production [27–29]. However, it is not clear whether this decrease is mirrored by an equivalent increase in PGE₃ production. EPA is a far less efficient substrate for COX than is arachidonic acid [65]. Also, EPA and another n-3 polyunsaturated fatty acid found in fish oil, docosahexaenoic acid, inhibit COX activity [66, 67], and markedly inhibit the cytokine-induced up-regulation of expression of the COX-2 gene [68, 69]. This range of effects means that the EPA-induced decrease in PGE₂ production may not be accompanied by increased PGE₃ production and that EPA may result in a decrease in the total amount of PGE produced. Indeed, Hawkes et al. [70] reported that incubation of adherent human mononuclear cells with EPA resulted in a significant decrease (50%) in PGE₂ production but no detectable PGE₃ production. A reduction in PGE₂ production without a concomitant increase in production of PGE₃ would result in a decreased bias towards the Th2 phenotype, and this might explain the recently reported epidemiological associations between oily fish or n-3 fatty acid intake and atopic disease [10, 71].

In summary, the current study demonstrates that PGE derived from n-6 and n-3 fatty acid precursors do not have differential effects on the production of Th1 and Th2 cytokines by mitogen-stimulated human whole-blood cultures. All three PGEs tested were able to down-regulate Th1 and up-regulate Th2 cytokine production. Thus, the suggested beneficial effects of increasing dietary n-3 and/or decreasing n-6 polyunsaturated fatty acid intakes on atopic disease may not be mediated through the substitution of n-6 fatty acid-derived PGE by less potent n-3 fatty acid-derived PGE. However, the mechanism may still be PGE-dependent, as the increased incorporation of n-3 polyunsaturated fatty acids into inflammatory cell membranes may decrease total PGE production.

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