

Prostaglandin-E2 Is a Potent Inhibitor of Human Interleukin 12 Production

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Summary

During human immunodeficiency virus infection and allergic diseases, characterized by a dominant T helper (Th) 2 response, overproduction of prostaglandin E2 (PGE2) is observed. In this paper we studied the effect of PGE2 on interleukin (IL)-12 synthesis, because this cytokine has been described to be essential in induction of Th1 responses. IL-12 synthesis was induced in monocytes that were stimulated with *Neisseria meningitidis*-derived lipopolysaccharide in whole blood cultures. PGE2 almost completely inhibited lipopolysaccharide induced IL-12 production, whereas IL-6 production was only partially inhibited by PGE2. In contrast, the production of IL-10 was approximately twofold enhanced at these conditions. The effects of PGE2 were due to its cAMP-inducing capacity, since they could be mimicked by other cAMP inducers. Recombinant human IL-10 also inhibited IL-12 and IL-6 production. However, the inhibitory effect of PGE2 on IL-12 production was independent of IL-10 since neutralizing anti-IL-10 antibodies were unable to reverse this inhibition. These results suggest that the capacity of an antigen to induce PGE2 synthesis may play a crucial role in the development of either a Th1 or Th2 response.

CD4⁺ T cells have been shown to differentiate into Th1 or Th2 cells, with different cytokine profiles and functions (1, 2). Th1 cells produce IL-2, IFN- γ , and TNF- β , activate macrophages, and cause delayed type of hypersensitivity reactions, whereas Th2 cells produce IL-4 and IL-5, cause eosinophilia, and are more specialized in providing B cell help for immunoglobulin production. The differentiation of T cells into either Th1 or Th2 cells can be influenced by the nature and concentration of the antigen as well as by its route of entry, the type of antigen-presenting cell, and soluble factors secreted by the APC and present in the microenvironment. One of these soluble factors secreted by monocytes, IL-12, is a major inducer of differentiation of T cells towards the Th1 type, while suppressing Th2 cytokine development (3-9). The capacity of IL-12 to stimulate growth and IFN- γ production in T cells and NK cells (4, 10-12) is probably the main reason for its Th1-inducing activity, although also IFN- γ -independent Th1 development has been suggested (7-9). Thus far, bacteria, bacterial products, and the protozoan *Toxoplasma gondii* have been described to induce production of IL-12 by macrophages and possibly B cells (3, 12-14). Less is known about the conditions leading to Th2 development. Another product of activated monocytes, PGE2, has been suggested to be an important regulatory factor in inducing Th2 responses (reviewed in 15). PGE2 and other cAMP-increasing agents affect T helper responses opposite to IL-12: the synthesis of Th1 cytokines (IL-2 and IFN γ) is much more sensitive to inhibition by PGE2/cAMP than

Th2 cytokine production (IL-4 and IL-5) (16-21). Because Th1 and Th2 cytokines negatively cross-regulate each other's production, the selective inhibition of Th1 cytokines by PGE2 could result in dominant Th2 responses. In this report we describe another mechanism of action of PGE2 on induction of Th2 responses, which represents an inhibition by PGE2 of the production of IL-12 by the APC. Since monocytes are able to secrete both PGE2 and IL-12 during antigen presentation, the balance of these two soluble factors will determine whether T cells differentiate into either Th1 or Th2 cells.

Materials and Methods

Antibodies and Reagents. Anti-IL-12 mAb C11.79 and C8.6 (12) were provided by Dr. G. Trinchieri (The Wistar Institute, Philadelphia, PA). The antibodies recognize both IL-12 p40 and the bioactive heterodimer p70, consisting of p40 and p35, as described (12). IL-12 p40 and p70 are produced in parallel by human PBMC, which is the result of p40 mRNA upregulation, whereas p35 mRNA expression is only minimally regulated (12, 13). Anti-IL-10 mAb B-T10 and B-N10 were produced at Innotherapie (Besançon, France). PGE2, 3-isobutyl-1-methylxanthine (IBMX) and *N*-2-*O*-dibutyryl-cAMP (DBcAMP) were obtained from Sigma Chemical Co. (St. Louis, MO). Recombinant human (rh) IL-10 was a kind gift from Dr. R. de Waal Malefijt (DNAX, Palo Alto, CA). *Neisseria meningitidis*-derived LPS was a gift from Dr. J. Poolman (RIVM, Bilthoven, The Netherlands).

Whole Blood Cultures. Whole blood was obtained by venipuncture from normal healthy donors in sodium heparin containing sterile blood collecting tubes (VT-100H tubes; Venoject, Terumo

Europe N.V., Leuven, Belgium). To prevent spontaneous production of cytokines by endotoxin or endotoxin like substances present in culture media, IMDM was ultrafiltered by means of a hollow fiber dialyzer (22) (Hemoflow F5; Fresenius A.G., Bad Homburg, Germany). Whole blood was 1:10 diluted in ultrafiltered IMDM, supplemented with 0.1% FCS, penicillin (100 IU/ml), streptomycin (100 µg/ml), and 50 IU/ml sodium heparin (Leo Pharmaceutical Products BV., Weesp, The Netherlands). Diluted whole blood was cultured in duplicate in 200-µl flat-bottomed culture plates (Nunc, Roskilde, Denmark). Supernatants were harvested after 18–20 h of culture and cytokine levels were determined.

Assays for Cytokines

IL-12 ELISA. mAb C11.79 was coated overnight (2 µg/ml in 0.1 M carbonate buffer, pH 9.6, 100 µl/well) on flat-bottomed microtiter plates (Maxisorb; Nunc). All subsequent incubations were in 100-µl vol at room temperature. The plates were washed twice with PBS, 0.02% (vol/vol) Tween 20 and incubated for 30 min with PBS, containing 2% (vol/vol) cow's milk as a blocking step. After washing, biotinylated purified mAb C8.6 was added (final concentration 0.25 µg/ml) together with IL-12-containing samples diluted in high performance ELISA (HPE) buffer (CLB, Amsterdam, The Netherlands) for 1.5 h. Thereafter the plates were washed five times and incubated with poly-streptavidin-horseradish peroxidase (poly-HRP; CLB), 1/10,000 diluted (according to the manufacturer's instruction) in PBS containing 2% (vol/vol) cow's milk for 0.5 h, washed, and developed with a solution of 100 µg/ml of 3,5,3',5'-tetramethylbenzidine (Merck, Darmstadt, Germany) with 0.003% (vol/vol) H₂O₂ in 0.11 M sodium acetate, pH 5.5 (100 µl/well). The reaction was stopped by adding an equal volume of 2 M H₂SO₄ to the wells. Plates were read at 450 nm in a Titertek Multiskan reader (Labsystems Multiskan Multisoft, Helsinki, Finland). Background absorbance at 540 nm was subtracted. rhIL-12 p70, provided by Dr. S. F. Wolf (Genetics Institute Inc., Cambridge, MA) was used as a standard, the detection limit was 4 pg/ml (twice the background).

IL-10 ELISA. The assay was performed identically to the IL-12 ELISA, except that the blocking step was omitted. For coating, mAb B-N10 was used at 0.5 µg/ml in PBS, and for detection, biotinylated mAb B-T10 was used at 0.125 µg/ml. rhIL10 was used as a standard. The detection limit was 10 pg/ml.

IL-6 ELISA. Procedures were identical to the IL-12 ELISA, with the following exceptions: the blocking step was eliminated; for coating, anti-IL-6 mAb CLB.IL6/16 was used at 1 µg/ml in PBS. Affinity-purified biotinylated polyclonal sheep anti-IL-6 was used for detection at 0.25 µg/ml diluted in PBS, 0.02% (vol/vol) Tween 20, 0.2% gelatin (PTG), and 1% normal sheep serum. Because full sensitivity of the IL-6 ELISA was not required, streptavidin-HRP (Amersham International, Little Chalfont, United Kingdom) diluted 1/1,000 in PTG was used instead of poly-HRP. rhIL-6 (23) was used as a standard. The detection limit was 16 pg/ml.

Results

In vitro IL-12 production by peripheral blood monocytes is induced by bacterial products such as LPS and *Staphylococcus aureus* Cowan I strain (SAC) (12). We used LPS from *N. meningitidis* to study the regulation of IL-12 production. In initial experiments the optimal conditions were established. 1:10 diluted peripheral blood was stimulated with various concentrations of LPS (Fig. 1). When instead of whole blood cultures PBMC were used, the production of IL-12 was ~10-

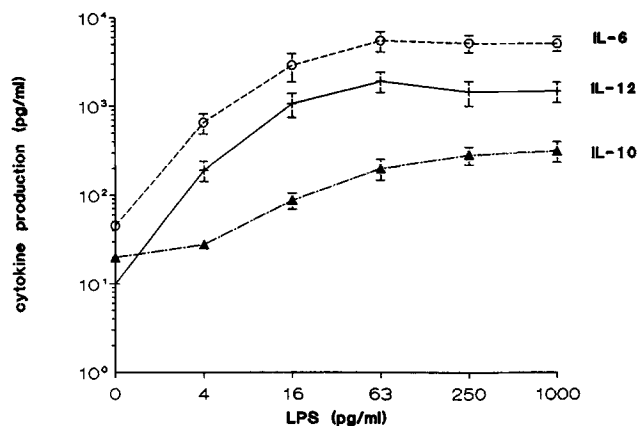


Figure 1. LPS dose-dependent production of IL-12, IL-6, and IL-10. 1:10 diluted whole blood was stimulated with indicated concentrations of LPS. After 18–20 h of culture cytokine levels were measured in the supernatants. Results are the mean production of four donors ± SE.

fold less on a per cell basis (not shown). Therefore we performed all experiments with 1:10 diluted whole blood. An incubation for 18–20 h turned out to be optimal (data not shown). IL-12 was induced by low concentrations of LPS, maximal production occurred at 63 pg LPS/ml, and remained constant up to 1,000 pg LPS/ml. In further experiments LPS was used at a concentration of 250 pg/ml. Concomitant with IL-12, other cytokines such as IL-1, IL-8, and TNF-α were produced. Here we concentrated on IL-12, IL-6, and IL-10 production (Fig. 1).

IL-12 and PGE2 have opposite effects on induction of Th1 versus Th2 responses. Therefore we investigated the influence of PGE2 on IL-12 production (Fig. 2). PGE2 turned out to be a very potent inhibitor of IL-12 production. At concentrations from 10⁻¹⁰ M on, PGE2 dose-dependently inhibited

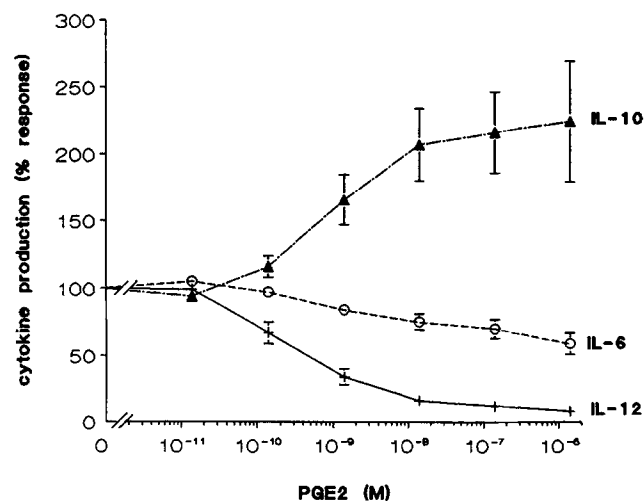


Figure 2. PGE2 inhibits IL-12 production. 1:10 diluted whole blood was stimulated with 250 pg LPS/ml. Graded concentrations of PGE2 were used as indicated. After 18 h of culture supernatants were harvested and cytokine levels were measured. Results are the mean production ± SE of five different donors. Mean LPS-induced IL-12 production was: 1,582 pg/ml; IL-6, 5,109 pg/ml; and IL-10, 286 pg/ml.

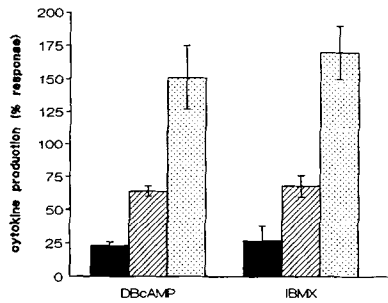


Figure 3. cAMP inhibits IL-12 production. 1:10 diluted blood was stimulated with 250 pg LPS/ml in the absence or presence of DBCAMP or IBMX at 50 μ M. After 18–20 h of culture cytokines were measured in the supernatants. Results are the mean \pm SE of four donors. Mean LPS-induced IL-12 production was: 1,520 pg/ml; IL-6, 4,412 pg/ml; and IL-10, 261 pg/ml. ■, IL-12; ▨, IL-6; ▩, IL-10.

the production of IL-12, yielding almost complete inhibition at 10^{-6} M PGE2 (8% of maximal response). In contrast, production of IL-6 was much less sensitive to PGE2. At 10^{-6} M PGE2, the production of IL-6 was inhibited by only 40%. Interestingly, the synthesis of IL-10 was approximately twofold increased at PGE2 concentrations ranging from 10^{-8} to 10^{-6} M.

Our next goal was to further analyze the mechanism of inhibition of IL-12 (and IL-6) production by PGE2. It is known that many effects of PGE2 are mediated via an increase in intracellular cAMP. We therefore analyzed whether cAMP could mimic the effects of PGE2. An increase of cAMP by DBCAMP indeed strongly inhibited IL-12 production and

to a lesser extent IL-6 production (Fig. 3). In agreement with the findings with PGE2, the synthesis of IL-10 was also enhanced by the addition of DBCAMP. Similar results were observed when the cAMP phosphodiesterase-inhibitor IBMX was used, which prevents the breakdown of intracellular cAMP.

IL-10 has been described as a strong inhibitor of IL-12 production by monocytes (13). Together with the observation that PGE2 enhanced IL-10 production (Fig. 2), these findings prompted us to investigate the possibility that the inhibitory effects of PGE2 and cAMP were mediated through the induction of IL-10. Exogenous IL-10 strongly inhibited the LPS-induced production of IL-12 and IL-6 (Fig. 4 A). This inhibition was prevented by the simultaneous addition of neutralizing anti-IL-10 (Fig. 4 B). In fact, IL-10 antibodies even enhanced the LPS-induced cytokine production, indicating that endogenously produced IL-10 inhibited the production of IL-12 and IL-6 in the absence of PGE2. However, inhibition of IL-12 production by PGE2 (Fig. 4 C) could not be reversed by anti-IL-10 (Fig. 4 D). This was not due to an incomplete neutralization of endogenous IL-10, because exogenous IL-10, at a concentration 10–40 times higher than that measured for endogenous IL-10, even more strongly inhibited IL-12 production than PGE2, which inhibition could be completely reversed by anti-IL-10. Therefore we concluded that the inhibitory effect of PGE2 on the production of IL-12 was largely independent of IL-10. In contrast, the inhibitory effect of PGE2 on LPS-induced production of IL-6 (Fig. 4 C) was reversed by anti-IL-10 (Fig. 4 D), indicating that this inhibition was indirect through enhancement of IL-10 production.

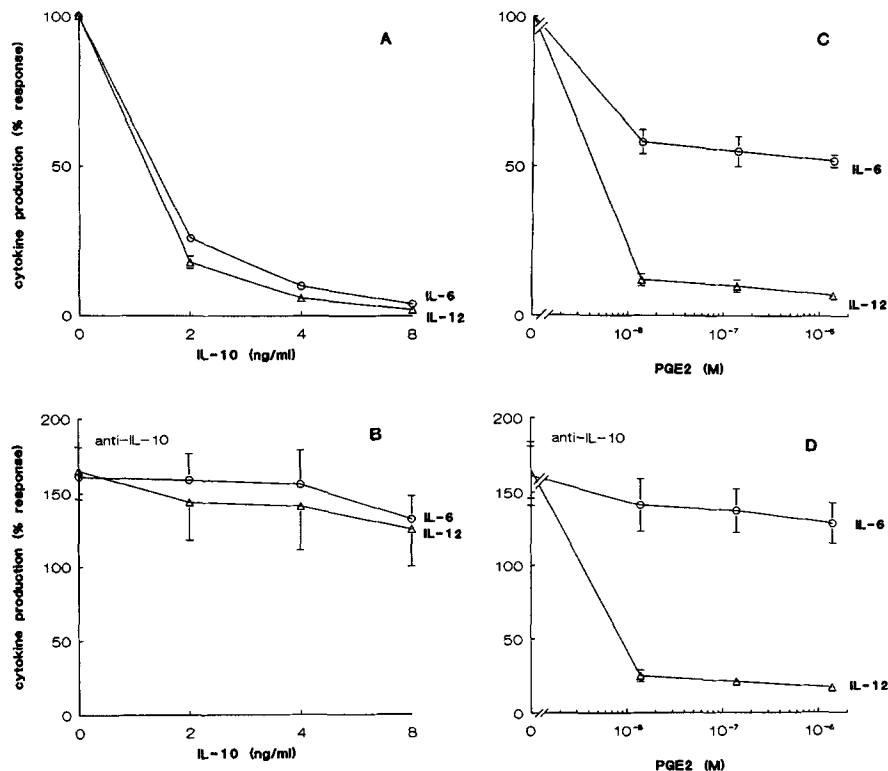


Figure 4. PGE2 inhibits IL-12 production independent of IL-10. 1:10 diluted whole blood cultures were stimulated with 250 pg LPS/ml. PGE2 and IL-10 were used at indicated concentrations, anti-IL-10 mAb BT-10 was used at 5 μ g/ml. After 20 h IL-12 and IL-6 production were measured. Results are the mean \pm SE of four donors. Mean LPS-induced IL-12 production was 1,681 pg/ml and mean IL-6 production was 3,032 pg/ml.

Discussion

In this report we show that PGE₂ inhibits the production of IL-12, in an IL-10-independent fashion, in whole blood cultures stimulated with LPS. The cells responsible for the production of cytokines in whole blood cultures were predominantly monocytes, because LPS, used as a stimulant, is a specific stimulus for monocytes, and anti-CD14 inhibited LPS-induced cytokine production. Elimination of CD14⁺ cells from PBMC revealed that IL-6 and IL-10 were produced by monocytes only. After CD14⁺ depletion, the remaining production of IL-12 was 25%, which was also inhibited by PGE₂. Depletion of CD19⁺ cells from PBMC did not result in reduction of cytokine production. Therefore we concluded that monocytes are the main producers of IL-12 in response to LPS. However, in whole blood cultures IL-12 production was at least 10-fold more efficient than in PBMC or adherent cells, whereas for IL-6 and IL-10 production no major differences were found in this respect. This was not due to the elimination of granulocytes, because these cells did not produce IL-12 (data not shown).

After binding to its receptor, PGE₂ stimulates adenylate cyclase, which results in elevated cAMP levels and protein kinase A activation (24). The increase of intracellular cAMP appeared to be responsible for the effect of PGE₂ since other cAMP-increasing agents like DBcAMP and IBMX similarly inhibited the production of IL-12. The inhibitory effect of PGE₂/cAMP on the production of cytokines was specific for IL-12, since no such effect was found for IL-8 (not shown) and IL-6. These two cytokines were both indirectly inhibited through enhancement of IL-10 production. IL-12 is a strong inducer of Th1 responses, *in vitro* as well as *in vivo* (3, 4, 6, 7). The fact that PGE₂ strongly inhibits the production of IL-12 implies a feedback mechanism at the level of the APC. Therefore two mechanisms of the PGE₂/cAMP pathway leading to Th2 responses can be identified: (a) a direct negative effect on the production of Th1 cytokines such as IL-2 and IFN- γ (16–21) and (b) downregulation of IL-12 production by monocytes (this report). This latter mechanism might be the most important effect of PGE₂ on T helper development, because production of IL-12 is two orders of magni-

tude more sensitive to inhibition by PGE₂ than Th1 cytokine secretion by T cells (17). Thus, the balance between the secretion of IL-12 and PGE₂ by the antigen-presenting monocyte will be crucial in determining whether a Th1 or Th2 response will dominate.

There is substantial evidence that this regulation of Th1 and Th2 responses indeed occurs at the level of the APC. It has been proposed that HIV infection induces a shift towards a Th2 response (25), which is believed to be mediated by the APC (26). This is particularly interesting, because PBMC cells from HIV-infected individuals were shown to produce diminished amounts of IL-12, due to a soluble factor different from IL-10 (27). Our experiments suggest that this factor might be PGE₂. This suggestion is supported by the fact that HIV infection of monocytes increases the production of PGE₂ (28, 29). In addition, PBMC from HIV-infected individuals have increased levels of cAMP (30). These observations together with our results suggest that in HIV-infected individuals, the diminished production of IL-12 is due to an increase of PGE₂. Moreover, in our hands the reduction of IL-12 production by PGE₂ was also independent of IL-10.

In atopic dermatitis and hyper-IgE syndrome patients, a Th2 response is responsible for enhanced IgE production. PBMC isolated from these patients also produced more PGE₂ than normal controls (31, 32). Although a diminished IL-12 production by monocytes from allergic patients has been reported yet, based on the above-mentioned results, we speculate that the production of IL-12 is indeed reduced in these patients.

The results presented in this report offer new possibilities for influencing T helper responses by modulating the secretion pattern of the antigen-presenting cell. We previously reported that the cytokine profile of T memory cells can be modulated during the first *in vitro* stimulation (33). These findings provide opportunities to treat patients with dominant Th2 responses by selectively inhibiting the synthesis of PGE₂ during vaccination or hyposensitization therapy as this would increase IL-12 production and cause a shift toward Th1 cytokine production.

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