Control of functional T helper cell polarization by dendritic cells

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Publication date
2002

Citation for published version (APA):
Limao Vieira, P. (2002). *Control of functional T helper cell polarization by dendritic cells*.

CHAPTER 5

Prostaglandin E$_2$ is a Selective Inducer of IL-12p40 Production and an Inhibitor of Bioactive IL-12p70 Heterodimer

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IL-12p70 heterodimer, composed of p35 and p40 subunits, is a major Th1-driving cytokine, promoting cell-mediated immunity. In contrast, IL-12p40 homodimer, secreted by APC in the absence of p35 expression, and free p40 monomer do not mediate IL-12 activity but act as IL-12 antagonists. We report that prostaglandin (PG)E$_2$, an inflammatory mediator with a previously known Th2-driving function, dose-dependently enhances the IL-12p40 mRNA expression and the secretion of IL-12p40 protein in human TNF-α–stimulated immature DC. This effect is selective and is not accompanied by the induction of IL-12p35 expression or by secretion of IL-12p70 heterodimer. Inability of TNF-α/PGE$_2$ to induce IL-12p70 was not compensated by IFN-γ, which strongly enhanced the LPS-induced IL-12p70 production. In addition to the selective induction of IL-12p40 in TNF-α–stimulated DC, PGE$_2$ inhibited the production IL-12p70 and IL-12p40 in DC stimulated with LPS or CD40 ligand. These data suggest an additional level of the Th2-promoting activity of PGE$_2$, via selective induction of IL-12p40. Selective induction of IL-12p40 and suppression of bioactive IL-12p70 may have negative impact on anti-cancer vaccination with PGE$_2$-matured DC.

INTRODUCTION

Bioactive IL-12p70 is a heterodimer composed of two subunits: p35 and p40 (1). While p35 is expressed in numerous cell types, p40 expression is more restricted, and determines the ability of a given cell-type to produce bioactive IL-12 (1). However, in the cells that are able of producing both IL-12 subunits, it is the p35 subunit, produced in much lower amounts than p40, that plays a role of a limiting factor and determines the amounts of bioactive IL-12p70 that is secreted (1, 2). IL-12p70 heterodimer is a major Th1-driving cytokine. In contrast, p40 homodimer, secreted by antigen presenting cells (APC) in the absence of p35 production, and to a lesser extent also the p40 monomer, were shown to act as antagonists of mouse and human IL-12 receptor (1, 3-5) and to inhibit IL-12–dependent immune functions in vitro and in vivo (6-13).

Bioactive IL-12p70 is produced by dendritic cells (DC) during their interaction with Th cells, as an result of CD40-CD40L interaction between these two cell types (14-18). While CD40-triggering alone provides a sufficient signal for the induction of IL-12p40 and several other cytokines, effective induction of IL-12 p70 depends on the presence of an additional signal that can be provided by at least two of the Th cell-produced cytokines, IFN-γ or IL-4 (17-19). DC also produce both IL-12p40 and IL-12p70 in response to many microbial stimuli, including LPS and SAC (17, 18).

Prostaglandin (PG)E$_2$, is a common inflammatory mediator with a Th2-driving role at several levels of the immune response. PGE$_2$ selectively impairs the production of IFN-γ and promotes the production of Th2 cytokines in murine and human Th clones, acting both directly (20, 21) and by inhibiting the responsiveness to IL-12 (22). The presence of PGE$_2$ during the priming of naive Th cells, drives their development into Th2 subset (23). At the level of APC, PGE$_2$ inhibits IL-12p70 production in CD40L- or LPS-stimulated cells (24, 25) and can induce a stable IL-12–deficient, Th2-inducing phenotype in maturing DC (25, 26). A similar IL-12–antagonistic and Th2-promoting activity is shared by other and cAMP-elevating agents, including β-adrenergic agonists (27), histamine (28), or cholera toxin (29).

In apparent contrast, PGE$_2$ was recently reported to synergize with TNF-α in the induction of at least IL-12p40 subunit production in DC, as determined by an IL-12p40–specific ELISA, sensitive for IL-12p70 heterodimer, p40 homodimer, and free p40 monomer (30). This led to the proposal that at the inflammatory sites PGE$_2$

may paradoxically play a role of an IL-12 inducer, supporting cell-mediated immunity. Since PGE$_2$ is known to enhance the TNF-α–induced DC maturation (26, 30, 31), its potential IL-12–inducing ability could be important for anti-cancer vaccination protocols utilizing DC matured in the presence of a "complete cytokine mix", consisting of TNF-α, IL-1β, IL-6 and PGE$_2$ (31).

Having in mind the opposite roles played by IL-12p70 and IL-12p40 and the different requirements for the induction of either factor in human DC (12, 17-19), we tested whether the enhancement of IL-12p40 production in TNF-α–activated DC by PGE$_2$ is accompanied by the induction of p35 gene transcription and by secretion of bioactive IL-12p70. Our results indicate that although PGE$_2$ participates in the induction of IL-12p40, this effect is selective and is not accompanied by the induction of p35 gene expression nor by the production of bioactive IL-12p70 heterodimer. In addition, PGE$_2$ suppresses the production of IL-12p70 induced by the "classical" inducers of cytokine production, LPS or CD40L.

MATERIALS AND METHODS

Generation of "tissue-type" immature CD1a$^+$CD83$^-$ DC

Monocytes were isolated from peripheral blood of healthy volunteers, and cultured at 5 x 10^5 cells/ml in IMDM with 10% FCS (Hyclone, Logan, UT) in the presence of rhuGM-CSF (500 U/ml; a gift of Schering-Plough, Uden, The Netherlands) and rhuIL-4 (250 U/ml; Strathman, Hannover, Germany) for 6 days, as described before (25,26). At day 6, the cultures consisted of uniformly HLA-DR$^+$ (L243; Becton Dickinson, San Jose, CA), CD83$^-$ (HB15; Immunotech, Marseille, France) immature DC, without detectable CD3$^+$ cells, as analyzed by FACScan (Becton Dickinson). Over 90% of the cells expressed high levels of CD1a (OKT6; Ortho, Beerse, Belgium).

Analysis of cytokine production

At day 6, DC were washed, counted and stimulated (4 x 10^4) in a final volume of 200 μl by rhuTNF-α (50 ng/ml, corresponding to 5 x 10^3 U/ml, PBH), LPS (250 ng/ml, Difco, Detroit, MI), or CD40L-transfected J558 plasmacytoma cells (J558-CD40L, 5 x 10^4 cells per well, a gift of Dr P. Lane, Birmingham, UK), which were previously shown to induce IL-12 p70 in IFN-γ-independent way (16), alone or in combination with PGE$_2$ (10^{-5} M - 10^{-9} M, as indicated; Sigma), or rhuIFN-γ (1000 U/ml; a gift of Dr

P. H. van der Meide, U-CyTech, Utrecht, The Netherlands), as indicated, for an additional period of 48 hours. The concentrations of cytokines in 48 hour supernatants were analyzed with specific solid phase sandwich ELISAs.

Cytokine measurements

IL-12p70 ELISA (sensitivity, 3 pg/ml) (27), was performed with use of p70-specific mAb 20C2 (a gift from Dr. M.K. Gately, Hoffmann-La Roche, Nutley, NJ) and p40-specific C8.6 mAb (a gift from Dr G. Trinchieri, The Wistar Institute, Philadelphia, PA). IL-12 p40-specific ELISA, recognizing p40 monomer, p40 homodimer, and p70 (p40 + p35) heterodimer was performed as described before (2, 25).

Analysis of p35 and p40 mRNA expression

DC (3×10^5 cells in 2 ml) were stimulated with the combination of TNF-α (50 ng/ml) and PGE$_2$ (10^{-6} M) in the presence of IFN-γ (1000 U/ml) or with LPS (250 ng/ml) and IFN-γ (1000 U/ml), as indicated. Unstimulated DC and DC exposed to IFN-γ alone were used as negative controls. Cells were lyzed after 6 hours and total RNA was isolated using the RNeasy Kit (Qiagen, Hilden, Germany). First strand cDNA was synthesized from the total amount of RNA using Moloney murine leukemia virus (mMLV)-derived reverse transcriptase (MBI fermentas, Vilnius, Lithuania). PCR amplification of p35 (3 ml of cDNA; 40 cycles) and p40 (2 ml of cDNA; 35 cycles) was carried out using the p35 and p40 primers (2), resulting in the products of 533 bp and 267 bp, respectively. PCR specific for human $\beta2\mu$ (1 ml cDNA, 35 cycles) was performed with specific primers (32), resulting in the product of 286 bp. Ethidium bromide-stained PCR products were analyzed on agarose gel by Eagle-Eye (Stratagene, La Jolla, CA) and SigmaGel software (SPSS Science, Jandel Scientific Software, Chicago, IL).

RESULTS

The induction of IL-12p40 mRNA and protein in DC by TNF-α and PGE$_2$ is selective and is not accompanied by the induction of IL-12p35 mRNA or bioactive IL-12p70 heterodimer

PGE$_2$ was previously shown to enhance the TNF-α–induced final maturation of DC (26, 30, 31), which was accompanied by the production of at least p40 subunit of

IL-12 (30). To test whether the production of p40 in these conditions is accompanied by the induction of IL-12p35 subunit and the secretion of bioactive IL-12p70, we analyzed the regulation of both IL-12 forms in TNF-α–activated DC by PGE$_2$.
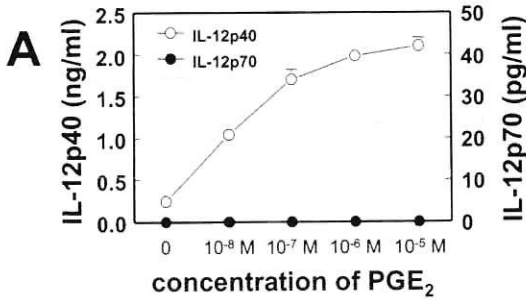
Figure 1. PGE$_2$ selectively enhances IL-12p40 production in TNFα-stimulated DC, but does not induce IL-12p70 production. (A) DC (4 x 10^4 cells in 0.2 ml) were stimulated with TNFα (50 ng/ml) and increasing concentrations of PGE$_2$ for 48 hours. The data are shown as mean (± SD) of triplicate cultures and represent one experiment of three that gave similar results. (B) DC (4 x 10^4 cells in 0.2 ml) were stimulated for 48 hours with one of the following stimuli: TNFα (50 ng/ml), PGE$_2$ (10^{-6} M), or their combination, or LPS (250 ng/ml) in the absence or in the presence of IFN-γ (1000 U/ml). The 48 hour culture supernatants were harvested and analyzed for IL-12p40 and IL-12p70 contents with specific ELISAs. The data are shown as mean (± SD) of triplicate cultures and represent one experiment of five that all gave similar results.
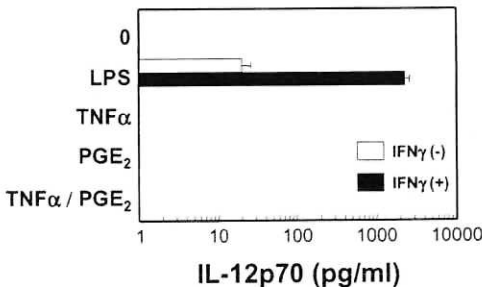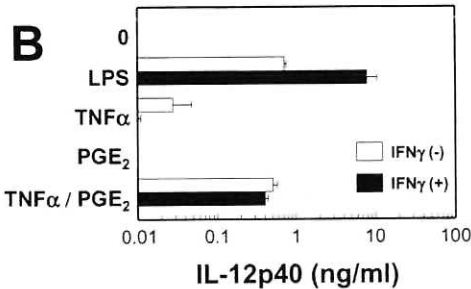
In accordance with the previous report, immature DC exposed to TNF-α (50 ng/ml) produced low, but clearly detectable amounts of IL-12p40, found in 48 hour supernatants (Fig.1A, B). Addition of increasing concentrations of PGE$_2$ to the

cultures of TNF-α–stimulated DC dose-dependently enhanced the IL-12p40 production. At all concentrations, the IL-12p40 induction was selective and was not accompanied by the induction of IL-12p70 heterodimer (Fig. 1A). Although the combination of TNF-α and PGE$_2$ was as effective as LPS (250 ng/ml) in the induction of IL-12p40 production, exclusively LPS, but neither TNF-α alone nor the combination of TNF-α and PGE$_2$ could induce IL-12p70 production (Fig. 1B).

Previously, we have shown that the physiologic levels of CD40 triggering, e.g. by CD40L-expressing Th cells, are sufficient to induce IL-12p40 production, but the efficient induction of IL-12p70 requires an additional IFN-γ–mediated or IL-4– mediated signal (18-20). Similarly, IFN-γ strongly enhances the IL-12p70 (and to a lesser extent IL-12p40) production in immature DC and monocytes stimulated by bacterial products (2, 18, 19, 25). Therefore, we tested if the selective inability of TNF-α and PGE$_2$ to induce IL-12p70 production can be overcome by IFN-γ. As expected (2, 18-20), IFN-γ (1000 U/ml) strongly enhanced the IL-12p70 (and IL-12p40) production by LPS-stimulated DC (Fig.1B). In contrast, the addition of IFN-γ to DC cultures stimulated either with TNF-α alone or with the combination of TNF-α and PGE$_2$, did not induce any detectable IL-12p70 production and suppressed the production of IL-12p40 (Fig. 1B).

Since the secretion of bioactive IL-12p70 requires the production of both IL-12 subunits: p35 and p40, we analyzed the induction of mRNA encoding either of these subunits. As shown in Fig. 2, both p35 and p40 expression was observed in DC stimulated with LPS and IFN-γ. In contrast, stimulation with TNF-α/PGE$_2$ and IFN-γ resulted with the selective induction of p40 in the absence of p35 induction. These results indicate that, even in the presence of IFN-γ, a powerful coinducer of IL-12 production, the combination of inflammatory factors TNF-α and PGE$_2$ does not induce the bioactive IL-12p70.
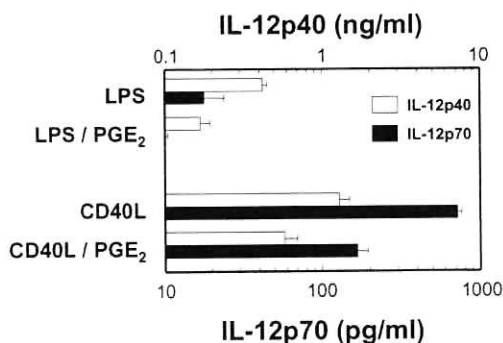
PGE$_2$ inhibits the production of both forms of IL-12 in DC stimulated with "classical IL-12 inducers", CD40L or LPS

To test whether PGE$_2$ differentially regulates the production of IL-12p40 and IL-12p70, also during the stimulation of DC with other stimuli, we added increasing concentrations of PGE$_2$ to DC cultures stimulated with two classical IL-12 inducers

known to induce both IL-12p40 and bioactive IL-12p70, LPS and CD40L-transfected J558 cells (J558-CD40L) that express very high levels of CD40L. As shown in figure 3, PGE$_2$ inhibited the production of both IL-12p70 and IL-12p40 production induced by either of these stimuli.

IFN-γ

--- --- LPS TNFα/PGE$_2$

β2μ

p40

p35
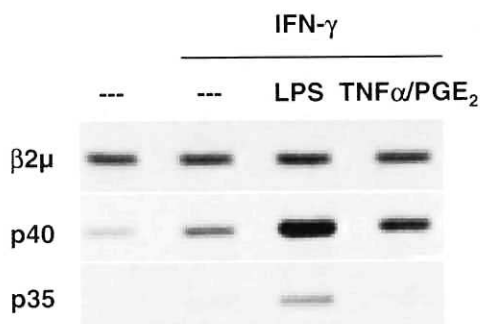
Figure 2. The combination of TNFa and PGE$_2$ induces IL-12p40 gene expression but fails to induce IL-12p35. DC (3 x 10^5 cells in 2 ml) were stimulated for 6 hours with one of the following stimuli: TNFα (50ng/ml) and PGE$_2$ (10^{-6} M) or LPS (250 ng/ml) in the presence of IFN-γ (1000 U/ml), as indicated, and lyzed for mRNA extraction. The expression of p35, p40 and β2μ was analyzed with RT-PCR (see Materials & Methods). The data shown are from a representative experiment of three performed.

IL-12p40 (ng/ml)

0.1 1 10

LPS

LPS / PGE$_2$

☐ IL-12p40
■ IL-12p70

CD40L

CD40L / PGE$_2$

10 100 1000

IL-12p70 (pg/ml)

Figure 3. PGE$_2$ inhibits the production of IL-12p70 and IL-12p40 in DC stimulated with CD40L or LPS. DC (4 x 10^4 cells in 0.2 ml) were stimulated with CD40L-transfected J558 cells (5 x 10^4 cells in 0.2 ml) or with LPS (250 ng/ml), either in the absence or in the presence of PGE$_2$ (10^{-6} M). The supernatants were harvested after 48 hours and analyzed for IL-12p40 and IL-12p70 contents with specific ELISAs. The data are shown as mean (± SD) of triplicate cultures and represent one experiment of four that all gave similar results.

DISCUSSION

The current demonstration that the PGE$_2$-assisted enhancement of IL-12p40 production in TNF-α–stimulated DC is selective and not accompanied by the induction of bioactive IL-12p70 heterodimer allows to resolve the current controversy concerning the role of PGE$_2$ in IL-12 regulation and underlines confirms the role of PGE$_2$ as a factor with a multiple Th2-driving activities.

PGE$_2$ is known to exert a Th2-promoting and IL-12–antagonistic activity via several distinct mechanisms, affecting both APC and Th cells. It suppresses the production of bioactive IL-12p70, both directly (24-26), and by inhibiting the Th cell production of IFN-γ (20, 21), an important co-inducer of IL-12 during DC-Th cell cocultures (17-19). PGE$_2$ also inhibits the responsiveness of T cells to IL-12 by downregulating the expression of IL-12 receptor (22). Another example of IL-12–antagonistic and Th2-driving function of PGE$_2$ is the induction of IL-10. Although in the current experiments we could not detect any significant enhancement of IL-10 production when PGE$_2$ was applied during the TNF-α– or LPS-induced DC maturation, nor the priming of maturing DC for elevated IL-10 production upon subsequent CD40L stimulation (data not shown), PGE$_2$ is known to enhance IL-10 production in mouse and human monocytes or when present at early stages of DC development (25, 35-40).

Human DC matured in the presence of PGE$_2$ show reduced IL-12–producing capacity, compared to mature DC obtained in the presence of high doses of IL-1β and TNF-α alone, and preferentially induce Th2 cytokines in naive Th cells (26). The observation that such IL-12–deficient and control DC showed the same expression of maturation-associated markers (26), together with the currently reported ability of PGE$_2$ to directly inhibit the IL-12 production in LPS- or CD40L-activated DC (Fig. 3), argue that the IL-12 suppressive activity of PGE$_2$ does not merely reflects its ability to accelerate DC maturation (a process associated with a decrease in IL-12–production (33, 34), but represents a specific IL-12–inhibitory function.

The presently described selective induction of IL-12p40 suggests the existence of an additional level of Th2-promoting and IL-12–antagonistic activity of PGE$_2$, mediated by p40 homodimer, or by free IL-12p40. Both in mouse and in human system, it was demonstrated that p40 homodimer and to a lesser extent also the product of its dissociation, free p40 monomer, can suppress the responsiveness to IL-12 by competitively inhibiting the IL-12 receptor binding (1, 3-5). This activity is very well pronounced in mouse where p40 homodimer exerts the antagonistic activity with an IC$_{50}$ of 1-10 ng/ml, while in humans at least 10-fold higher concentrations are required (3-5). Mouse p40 homodimer was shown to effectively antagonize IL-12 activity in vivo, as a factor rescuing the animals from lethal LPS-induced shock and suppressing the Th1-dominated inflammatory responses in several models of chronic inflamation, transplantation, and cancer (6-12). In humans, high levels of IL-12p40 in

peritoneal fluid were postulated to play an IL-12–antagonistic role in endometriosis, as a factor locally inhibiting the activity of NK cells (13). Unfortunately, poor stability of human p40 homodimer (1) constitutes a serious obstacle in analyzing its physiologic role, and its potential therapeutic in vivo use in transplantation and autoimmune diseases. For the same reason, we could not determine if the PGE$_2$-induced IL-12p40 was released from DC as p40 homodimer or p40 monomer, a less potent IL-12 antagonist (1).

Current demonstration of the selective induction of inactive/antagonistic IL-12p40 argues against the proposed Th1-promoting role for PGE$_2$ at the inflammatory sites (30). On the contrary, selective induction of IL-12p40 by PGE$_2$ is likely to contribute to selective suppression of Th1-type responses in chronic inflammation, and may play a role in the immune deviation induced by the PGE$_2$-producing tumors (35-40). Although PGE$_2$ enhances the expression of costimulatory molecules on DC and increases their ability to stimulate and CD4$^+$ and CD8$^+$ T cells (26, 30, 31), the ability of PGE$_2$ to induce IL-12p40 production and to suppress the IL-12p70-producing ability of DC (25, 26) may impair the tumoricidal functions of Th1, NK cells and CTLs. Multiple levels of IL-12–antagonism of PGE$_2$ may have negative impact on the effectiveness of immunotherapeutic protocols that use PGE$_2$ in combination with IL-1β, TNF-α, and IL-6 to induce mature DC for anti-cancer vaccination (31). It remains to be tested whether the benefit of an enhanced stimulatory capacity of PGE$_2$-matured DC is sufficient to offset the IL-12 antagonistic effects of PGE$_2$.

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