UvA-DARE (Digital Academic Repository)

Prostaglandin E2 is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer

Kalinski, P.; Limao Vieira, P.; Schuitemaker, J.H.N.; de Jong, E.C.; Kapsenberg, M.L.

DOI

[10.1182/blood.V97.11.3466](https://doi.org/10.1182/blood.V97.11.3466)

Publication date

2001

Published in

Blood

[Link to publication](#)

Citation for published version (APA):

Kalinski, P., Limao Vieira, P., Schuitemaker, J. H. N., de Jong, E. C., & Kapsenberg, M. L. (2001). Prostaglandin E2 is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer. *Blood*, 97(11), 3466-3469. <https://doi.org/10.1182/blood.V97.11.3466>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Prostaglandin E₂ is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer

Paweł Kaliński, Pedro L. Vieira, Joost H. N. Schuitemaker, Esther C. de Jong, and Martien L. Kapsenberg

Interleukin-12 p70 (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. Here it is reported that prostaglandin E₂ (PGE₂), 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 tumor necrosis factor- α (TNF α)-stimulated immature dendritic cells (DCs). 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 /PGE₂ to induce IL-12p70 was not compensated by interferon gamma (IFN γ), which strongly enhanced the lipopolysaccharide (LPS)-induced IL-12p70 production. In addition to the selective induction of IL-12p40 in TNF α -stimulated DCs, PGE₂ inhibited the production of IL-12p70 and

IL-12p40 in DCs stimulated with LPS or CD40 ligand. These data suggest an additional level of the Th2-promoting activity of PGE₂, via selective induction of IL-12p40. Selective induction of IL-12p40 and suppression of bioactive IL-12p70 may have negative impact on anticancer vaccination with PGE₂-matured DCs. (Blood. 2001;97:3466-3469)

© 2001 by The American Society of Hematology

Introduction

Bioactive interleukin-12 p70 (IL-12p70) is a heterodimer composed of 2 subunits: p35 and p40.¹ Although 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 capable of producing both IL-12 subunits, it is the p35 subunit, produced in much lower amounts than p40, that plays the role of a limiting factor and determines the amount 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 (APCs) in the absence of p35 production, and to a lesser extent the p40 monomer, were shown to act as antagonists of mouse and human IL-12 receptors^{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 (DCs) during their interaction with Th cells, as a result of CD40-CD40L interaction between these 2 cell types.¹⁴⁻¹⁸ Although CD40-triggering alone provides a sufficient signal for the induction of IL-12p40 and several other cytokines, effective induction of IL-12p70 depends on the presence of an additional signal that can be provided by at least 2 of the Th cell-produced cytokines, interferon gamma (IFN γ) or IL-4.¹⁷⁻¹⁹ DCs also produce both IL-12p40 and IL-12p70 in response to many microbial stimuli, including lipopolysaccharide (LPS) and *Staphylococcus aureus* Cowan strain I (SAC).^{17,18}

Prostaglandin E₂ (PGE₂), is a common inflammatory mediator

with a Th2-driving role at several levels of immune response. PGE₂ selectively impairs the production of IFN γ and promotes the production of Th2 cytokines in murine and human Th clones, acting directly^{20,21} and by inhibiting the responsiveness to IL-12.²² The presence of PGE₂ during the priming of naive Th cells, drives their development into Th2 subset.²³ At the level of APCs, PGE₂ 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 DCs.^{25,26} A similar IL-12 antagonistic and Th2-promoting activity is shared by other and cyclic adenosine monophosphate (cAMP)-elevating agents, including β -adrenergic agonists,²⁷ histamine,²⁸ or cholera toxin.²⁹

In apparent contrast, PGE₂ was recently reported to synergize with tumor necrosis factor- α (TNF α) in the induction of at least IL-12p40 subunit production in DCs, as determined by an IL-12p40-specific enzyme-linked immunosorbent assay (ELISA), sensitive for IL-12p70 heterodimer, p40 homodimer, and free p40 monomer.³⁰ This led to the proposal that at the inflammatory sites PGE₂ may paradoxically play the role of an IL-12 inducer, supporting cell-mediated immunity. Since PGE₂ is known to enhance the TNF α -induced DC maturation,^{26,30,31} its potential IL-12-inducing ability could be important for anticancer vaccination protocols utilizing DCs matured in the presence of a "complete cytokine mix," consisting of TNF α , IL-1 β , IL-6, and PGE₂.³¹

Having in mind the opposite roles played by IL-12p70 and IL-12p40 and the different requirements for the induction of

From the Department of Cell Biology and Histology and the Department of Dermatology, Academic Medical Center, University of Amsterdam, The Netherlands.

Submitted October 20, 2000; accepted January 5, 2001.

Supported by Fundação para a Ciência e a Tecnologia, Lisbon, Portugal (grant no. PRAXIS XXI/BD/9195/96 to P.L.V.).

Correspondence: Paweł Kaliński, Department of Surgery, University of Pittsburgh, W1540 BST, 200 Lothrop St, Pittsburgh, PA 15261; e-mail:

kalinski@msx.upmc.edu, or Martien L. Kapsenberg, Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands; e-mail: m.l.kapsenberg@amc.uva.nl.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2001 by The American Society of Hematology

either factor in human DCs,^{1,2,17-19} we tested whether the enhancement of IL-12p40 production in TNF α -activated DCs by PGE₂ is accompanied by the induction of p35 gene transcription and by secretion of bioactive IL-12p70. Our results indicate that although PGE₂ 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, PGE₂ 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⁻ DCs

Monocytes were isolated from peripheral blood of healthy volunteers and cultured at 5×10^5 cells/mL in Iscoves modified Dulbecco medium (IMDM) with 10% fetal calf serum (FCS) (Hyclone, Logan, UT) in the presence of rhu granulocyte-macrophage-colony-stimulating factor (GM-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 elsewhere.^{25,26} At day 6, the cultures consisted of uniformly HLA-DR⁺ (L243; Becton Dickinson, San Jose, CA), CD83⁻ (HB15; Immunotech, Marseille, France) immature DCs, without detectable CD3⁺ cells, as analyzed by FACScan (Becton Dickinson). Over 90% of the cells expressed high levels of CD1a (OKT6; Ortho, Beers, Belgium).

Analysis of cytokine production

At day 6, DCs were washed, counted, and stimulated (4×10^4) in a final volume of 200 μ L by rhuTNF α (50 ng/mL, corresponding to 5×10^3 U/mL, Pharma Biotechnologie Hannover), LPS (250 ng/mL, Difco, Detroit, MI), or CD40L-transfected J558 plasmacytoma cells (J558-CD40L, 5×10^4 cells per well) (a gift of Dr P. Lane, Birmingham, United Kingdom), which were previously shown to induce IL-12 p70 in an IFN γ -independent way,¹⁶ alone or in combination with PGE₂ (10^{-5} M – 10^{-9} M, as indicated; Sigma, St Louis, MO), 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-12p40-specific ELISAs, recognizing p40 monomer, p40 homodimer, and p70 (p40 + p35) heterodimer were performed as described elsewhere.^{2,25}

Analysis of p35 and p40 mRNA expression

DCs (3×10^5 cells in 2 mL) were stimulated with the combination of TNF α (50 ng/mL) and PGE₂ (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 DCs and DCs exposed to IFN γ alone were used as negative controls. Cells were lysed 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 (RT) (MBI fermentas, Vilnius, Lithuania). Polymerase chain reaction (PCR) amplification of p35 (3:1 of cDNA; 40 cycles) and p40 (2:1 of cDNA; 35 cycles) was carried out using the p35 and p40 primers,² resulting in the products of 533 base pair (bp) and 267 bp, respectively. PCR specific for human b2m (1:1 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 DCs by TNF α and PGE₂ is selective and is not accompanied by the induction of IL-12p35 mRNA or bioactive IL-12p70 heterodimer

PGE₂ was previously shown to enhance the TNF α -induced final maturation of DCs,^{26,30,31} which was accompanied by the production of at least a p40 subunit of IL-12.³⁰ To test whether the production of p40 in these conditions is accompanied by the induction of an IL-12p35 subunit and the secretion of bioactive IL-12p70, we analyzed the regulation of both IL-12 forms in TNF α -activated DCs by PGE₂.

In accordance with the previous report, immature DCs exposed to TNF α (50 ng/mL) produced low, but clearly detectable, amounts of IL-12p40, found in 48-hour supernatants (Figure 1A,B). Addition of increasing concentrations of PGE₂ to the cultures of TNF α -stimulated DCs 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 (Figure 1A). Although the combination of TNF α and PGE₂ 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 PGE₂, could induce IL-12p70 production (Figure 1B).

Previously, we have shown that the physiologic levels of CD40 triggering, by CD40L-expressing Th cells, for example, 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 DCs and monocytes stimulated by bacterial products.^{2,18,19,25} Therefore, we

Figure 1. PGE₂ selectively enhances IL-12p40 production in TNF α -stimulated DCs, but does not induce IL-12p70 production. (A) DCs (4×10^4 cells in 0.2 mL) were stimulated with TNF α (50 ng/mL) and increasing concentrations of PGE₂ for 48 hours. The data are shown as mean ($\pm$ SD) of triplicate cultures and represent one experiment of 3 that gave similar results. Δ , IL-12p40; $\bullet$, IL-12p70. (B) DCs (4×10^4 cells in 0.2 mL) were stimulated for 48 hours with one of the following stimuli: TNF α (50 ng/mL), PGE₂ (10^{-6} M), or their combination, or LPS (250 ng/mL) in the absence ($\square$) or presence ($\blacksquare$) 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 ($\pm$ SD) of triplicate cultures and represent one experiment of 5 that all gave similar results.

tested if the selective inability of $\text{TNF}\alpha$ and PGE_2 to induce IL-12p70 production can be overcome by $\text{IFN}\gamma$. As expected,^{2,18-20} $\text{IFN}\gamma$ (1000 U/mL) strongly enhanced the IL-12p70 (and IL-12p40) production by LPS-stimulated DCs (Figure 1B). In contrast, the addition of $\text{IFN}\gamma$ to DC cultures stimulated either with $\text{TNF}\alpha$ alone or with the combination of $\text{TNF}\alpha$ and PGE_2 , did not induce any detectable IL-12p70 production and suppressed the production of IL-12p40 (Figure 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 Figure 2, both p35 and p40 expression were observed in DCs stimulated with LPS and $\text{IFN}\gamma$. In contrast, stimulation with $\text{TNF}\alpha/\text{PGE}_2$ and $\text{IFN}\gamma$ resulted with the selective induction of p40 in the absence of p35 induction. These results indicate that, even in the presence of $\text{IFN}\gamma$, a powerful coinducer of IL-12 production, the combination of inflammatory factors $\text{TNF}\alpha$ and PGE_2 does not induce the bioactive IL-12p70.

PGE_2 inhibits the production of both forms of IL-12 in DCs stimulated with the "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 DCs with other stimuli, we added increasing concentrations of PGE_2 to DC cultures stimulated with 2 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.

Discussion

The current demonstration that the PGE_2 -assisted enhancement of IL-12p40 production in $\text{TNF}\alpha$ -stimulated DCs 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 confirms the role of PGE_2 as a factor with a multiple Th2-driving activities.

PGE_2 is known to exert Th2-promoting and IL-12-antagonistic activity via several distinct mechanisms, affecting both APCs and Th cells. It suppresses the production of bioactive IL-12p70, both directly²⁴⁻²⁶ and by inhibiting the Th-cell production of $\text{IFN}\gamma$,^{20,21} an important coinducer of IL-12 during DC-Th cell cocultures.¹⁷⁻¹⁹

Figure 2. The combination of $\text{TNF}\alpha$ and PGE_2 induces IL-12p40 gene expression but fails to induce IL-12p35. DCs (3×10^5 cells in 2 mL) were stimulated for 6 hours with one of the following stimuli: $\text{TNF}\alpha$ (50 ng/mL) and PGE_2 (10^{-6} M) or LPS (250 ng/mL) in the presence of $\text{IFN}\gamma$ (1000 U/mL), as indicated, and lysed for mRNA extraction. The expression of p35, p40, and $\beta 2m$ was analyzed with RT-PCR (see "Materials and methods"). The data shown are from a representative experiment of 3 performed.

Figure 3. PGE_2 inhibits the production of IL-12p70 and IL-12p40 in DCs stimulated with CD40L or LPS. DCs (4×10^4 cells in 0.2 mL) were stimulated with CD40L-transfected J558 cells (5×10^4 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 ($\pm$) of triplicate cultures and represent one experiment of 4 that all gave similar results.

PGE_2 also inhibits the responsiveness of T cells to IL-12 by downregulating the expression of the IL-12 receptor.²² Another example of IL-12-antagonistic and Th2-driving function of PGE_2 is the induction of IL-10. PGE_2 is known to enhance IL-10 production in mouse and human monocytes or in DC, when present at early stages of their development.^{25,35-40} However, in the current experiments, PGE_2 did not enhance IL-10 production during the $\text{TNF}\alpha$ - or LPS-induced DC maturation, and it did not prime maturing DC for elevated IL-10 production upon subsequent CD40L stimulation (data not shown).

Human DCs matured in the presence of PGE_2 showed reduced IL-12-producing capacity, compared with mature DCs obtained in the presence of high doses of IL-1 β and $\text{TNF}\alpha$ alone, and preferentially induce Th2 cytokines in naive Th cells.²⁶ The observation that such IL-12-deficient and control DCs 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 DCs (Figure 3), argue that the IL-12 suppressive activity of PGE_2 does not merely reflect 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 systems, 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 mice where p40 homodimer exerts the antagonistic activity with an IC_{50} of 1-10 ng/mL, whereas in humans concentrations at least 10-fold higher 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 inflammation, 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 natural killer (NK) cells.¹³ Unfortunately, poor stability of human p40 homodimer¹ 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 DCs as p40 homodimer or as 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 PGE₂ at the inflammatory sites.³⁰ On the contrary, selective induction of IL-12p40 by PGE₂ 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 PGE₂-producing tumors.³⁵⁻⁴⁰ Although PGE₂ enhances the expression of costimulatory molecules on DCs and increases their ability to

stimulate CD4⁺ and CD8⁺ T cells,^{26,30,31} the ability of PGE₂ to induce IL-12p40 production and to suppress the IL-12p70-producing ability of DCs^{25,26} may impair the tumoricidal functions of Th1, NK cells, and cytotoxic T lymphocytes. Multiple levels of IL-12 antagonism of PGE₂ may have a negative impact on the effectiveness of immunotherapeutic protocols that use PGE₂ in combination with IL-1 β , TNF α , and IL-6 to induce mature DCs for anticancer vaccination.³¹ It remains to be tested whether the benefit of an enhanced stimulatory capacity of PGE₂-matured DCs is sufficient to offset the IL-12 antagonistic effects of PGE₂.

References

- Gately MK, Renzetti LM, Magram J, et al. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol*. 1998;16:495-521.
- Snijders A, Hilken CMU, van der Pouw Kraan TCTM, Engel M, Aarden L, Kapsenberg ML. Regulation of bioactive IL-12 production in lipopolysaccharide-stimulated human monocytes is determined by the expression of the p35 subunit. *J Immunol*. 1996;156:1207-1212.
- Mattner F, Fischer S, Guckes S, et al. The interleukin-12 subunit p40 specifically inhibits effects of the interleukin-12 heterodimer. *Eur J Immunol*. 1993;23:2202-2208.
- Ling P, Gately MK, Gubler U, et al. Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. *J Immunol*. 1995;154:116-127.
- Gillessen S, Carvajal D, Ling P, et al. Mouse interleukin-12 (IL-12) p40 homodimer: a potent IL-12 antagonist. *Eur J Immunol*. 1995;25:200-206.
- Gately MK, Carvajal DM, Connaughton SE, et al. Interleukin-12 antagonist activity of mouse interleukin-12 p40 homodimer in vitro and in vivo. *Ann New York Acad Sci*. 1996;795:1-12.
- Kato K, Shimozato O, Hoshi K, et al. Local production of the p40 subunit of interleukin 12 suppresses T-helper 1-mediated immune responses and prevents allogeneic myoblast rejection. *Proc Natl Acad Sci U S A*. 1996;93:9085-9089.
- Heinzel FP, Hujer AM, Ahmed FN, Renko RM. In vivo production and function of IL-12 p40 homodimers. *J Immunol*. 1997;158:4381-4388.
- Rothe H, O'Hara RM Jr, Martin S, Kolb H. Suppression of cyclophosphamide induced diabetes development and pancreatic Th1 reactivity in NOD mice treated with the interleukin (IL)-12 antagonist IL-12(p40)2. *Diabetologia*. 1997;40:641-646.
- Chen L, Chen D, Block E, O'Donnell M, Kufe DW, Clinton SK. Eradication of murine bladder carcinoma by intratumor injection of a bicistronic adenoviral vector carrying cDNAs for the IL-12 heterodimer and its inhibition by the IL-12 p40 subunit homodimer. *J Immunol*. 1997;159:351-359.
- Mattner F, Ozmen L, Podlaski FJ, et al. Treatment with homodimeric interleukin-12 (IL-12) p40 protects mice from IL-12-dependent shock but not from tumor necrosis factor alpha-dependent shock. *Infect Immun*. 1997;65:4734-4737.
- Schmidt C, Brijs L, Cliquet P, De Baetselier P. Increased IL-12 P40 homodimer secretion by spleen cells during in vivo growth of the BW-19 T cell hybridoma accompanies suppression of natural immunity. *Int J Canc*. 1998;77:460-466.
- Mazzeo D, Vigano P, Di Blasio AM, Sinigaglia F, Vignali M, Panina-Bordignon P. Interleukin-12 and its free p40 subunit regulate immune recognition of endometrial cells: potential role in endometriosis. *J Clin Endocrinol Metab*. 1998;83:911-916.
- Kelsall BL, Stuber E, Neurath M, Strober W. Interleukin-12 production by dendritic cells. The role of CD40-CD40L interactions in Th1 T-cell responses. *Ann New York Acad Sci*. 1996;795:116-126.
- Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med*. 1996;184:747-752.
- Koch F, Stanzi U, Jennewein P, et al. High level IL-12 production by murine dendritic cells: up-regulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J Exp Med*. 1996;184:741-746.
- Hilken CMU, Kaliński P, de Boer M, Kapsenberg ML. Human dendritic cells require exogenous interleukin-12-inducing factors to direct the development of naive T-helper cells toward the Th1 phenotype. *Blood*. 1997;90:1920-1926.
- Snijders A, Kaliński P, Hilken CMU, and Kapsenberg ML. High-level IL-12 production by human dendritic cells requires 2 signals. *Int Immunol*. 1998;10:1593-1598.
- Kaliński P, Smits H, Schuitemaker JHN, et al. IL-4 is a mediator of IL-12p70 induction by human Th2 cells: reversal of polarized Th2 phenotype by dendritic cells. *J Immunol*. 2000;165:1877-1881.
- Betz M, Fox BS. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J Immunol*. 1991;146:108-113.
- Snijderwint FG, Kaliński P, Wierenga EA, Bos JD, Kapsenberg ML. Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J Immunol*. 1993;150:5321-5329.
- Wu CY, Wang K, McDyer JF, Seder RA. Prostaglandin E2 and dexamethasone inhibit IL-12 receptor expression and IL-12 responsiveness. *J Immunol*. 1998;161:2723-2730.
- Demeure CE, Yang LP, Desjardins C, Raynaud P, Delespessie G. Prostaglandin E2 primes naive T cells for the production of anti-inflammatory cytokines. *Eur J Immunol*. 1997;27:3526-3531.
- van der Pouw Kraan TC, Boeijs LC, Smeenk RJ, Wijdenes J, Aarden LA. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J Exp Med*. 1995;181:775-779.
- Kaliński P, Hilken CM, Snijders A, Snijderwint FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol*. 1997;159:28-35.
- Kaliński P, Schuitemaker JH, Hilken CM, Kapsenberg ML. Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J Immunol*. 1998;161:2804-2809.
- Panina-Bordignon P, Mazzeo D, Lucia PD, et al. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. *J Clin Invest*. 1997;100:1513-1519.
- van der Pouw Kraan TC, Snijders A, Boeijs LC, et al. Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. *J Clin Invest*. 1998;102:1866-1873.
- Braun MC, He J, Wu CY, Kelsall BL. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor beta1 and beta2 chain expression. *J Exp Med*. 1999;189:541-552.
- Rieser C, Bock G, Klocker H, Bartsch G, Thurnher M. Prostaglandin E2 and tumor necrosis factor alpha cooperate to activate human dendritic cells: synergistic activation of interleukin 12 production. *J Exp Med*. 1997;186:1603-1608.
- Jonuleit H, Kuhn U, Muller G, et al. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *Eur J Immunol*. 1997;27:3135-3142.
- de Waal Malefyt R, Figdor CG, Huijbens R, et al. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. *J Immunol*. 1993;151:6370-6381.
- Kaliński P, Schuitemaker JH, Hilken CM, Wierenga EA, Kapsenberg ML. Final maturation of dendritic cells is associated with impaired responsiveness to IFN-gamma and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. *J Immunol*. 1999;162:3231-3236.
- Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of Th1, Th2 and nonpolarized T cells. *Nature Immunol*. 2000;1:3111-3116.
- Strassmann G, Patil-Koota V, Finkelman F, Fong M, Kambayashi T. Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2. *J Exp Med*. 1994;180:2365-2370.
- Kambayashi T, Alexander HR, Fong M, Strassmann G. Potential involvement of IL-10 in suppressing tumor-associated macrophages: colon-26-derived prostaglandin E2 inhibits TNF-alpha release via a mechanism involving IL-10. *J Immunol*. 1995;154:3383-3390.
- Huang M, Sharma S, Mao T, Dubinett SM. Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. *J Immunol*. 1996;157:5512-5520.
- Handel-Fernandez ME, Cheng X, Herbert LM, Lopez DM. Down-regulation of IL-12, not a shift from a T helper-1 to a T helper-2 phenotype, is responsible for impaired IFN-gamma production in mammary tumor-bearing mice. *J Immunol*. 1997;158:280-286.
- Huang M, Stolina M, Sharma S, et al. Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. *Canc Res*. 1998;58:1208-1216.
- Stolina M, Sharma S, Lin Y, et al. Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. *J Immunol*. 2000;164:361-370.