Brief Definitive Report

Prostaglandin E2 and Tumor Necrosis Factor α Cooperate to Activate Human Dendritic Cells: Synergistic Activation of Interleukin 12 Production

By Claudia Rieser,* Günther Böck,‡ Helmut Klocker,* Georg Bartsch,* and Martin Thurnher*

*From the *Department of Urology, and the ‡Institute for General and Experimental Pathology, University of Innsbruck, A-6020 Innsbruck, Austria*

Summary

Interleukin (IL)-12 is a proinflammatory cytokine that contributes to innate resistance and to the development of antigen-specific T cell responses. Among other effects, prostaglandin E2 (PGE2) inhibits the production of IL-12 by macrophages activated with lipopolysaccharide (LPS). Here we investigated the effects of PGE2 on human dendritic cells (DCs) which develop in the presence of GM-CSF and IL-4. We demonstrate that in the absence of LPS, PGE2 dose dependently stimulated the production of IL-12 by DCs. Although PGE2 alone stimulated the production of low amounts of IL-12 only, it synergized with tumor necrosis factor (TNF)-α to induce high levels of IL-12 production by DCs. Addition of TNF-α in the absence of PGE2 had no effect on IL-12 production. Conversely, in the presence of LPS, PGE2 inhibited IL-12 production by DCs in a dose-dependent manner. The combination of PGE2 and TNF-α efficiently silenced mannose receptor–mediated endocytosis in DCs and readily induced neo-expression of the CD83 antigen. In addition, the expression of various surface antigens such as major histocompatibility complex class I and II, adhesion, as well as costimulatory molecules was upregulated by this treatment. The effects of PGE2 on IL-12 synthesis and CD83 expression could be mimicked by dibutyryl-cAMP and forskolin, indicating that they were due to the intracellular elevation of cAMP levels. DC treated with PGE2 and TNF-α were most potent in stimulating allogeneic T cell proliferation. Our data demonstrate that PGE2 contributes to the maturation of human DCs and that PGE2 can be a potent enhancer of IL-12 production by human DCs.

Tissue injury induces an acute inflammatory response mediated by components of innate resistance such as macrophages which release proinflammatory factors (1). The local inflammatory response at the site of injury is characterized by the development of erythema, edema, and hyperalgesia (2). In addition to several vasoactive factors such as histamine, bradykinin, and nitric oxide (3, 4), prostaglandins (PGs) contribute to this process. The therapeutic benefit of nonsteroidal antiinflammatory drugs such as aspirin or indomethacin in inflammatory diseases is based on the inhibitory effects of these drugs on the synthesis of PGs (5). The importance of PGs in the inflammatory process has also been demonstrated in a rat model of carrageenan-induced paw inflammation (2). In this model, the selective neutralization of PGE2 by an antibody prevented the carrageenan-induced development of tissue edema and hyperalgesia. However, injection of PG into the skin causes minimal swelling demonstrating that PGs on their own have little inflammatory capacity. In contrast, in the presence of other mediators, PGs can synergistically amplify the local inflammatory response (6, 7). In contrast to these proinflammatory activities, PGs are also known to inhibit the production of proinflammatory cytokines by macrophages activated with LPS. PGE2 has been shown to inhibit the LPS-induced human IL-12 production in whole blood cultures (8) and the LPS-stimulated murine TNF-α production in cultured macrophages (9), supporting the view that PGE2 acts as a suppressor of APC.

APCs link the inflammatory response to an Ag-specific immunological response (1). Dendritic cells (DCs) are the most potent APCs (10). DCs are highly responsive to inflammatory stimuli such as bacterial LPS and TNF-α which induce a series of phenotypic and functional changes in DCs. These changes, which have been collectively referred to as DC maturation (11), include the silencing of Ag uptake by endocytosis (12), the upregulation of surface molecules related to T cell activation (12), and the active production of a number of cytokines including TNF-α and

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/11/1603/06 $2.00
Volume 186, Number 9, November 3, 1997 1603–1608
http://www.jem.org

IL-12 (13, 14). Upon local accumulation of TNF-α, DCs migrate to the T cell areas of secondary lymphoid organs to activate Ag-specific T cells (15).

In the present work, we have investigated the effects of PGE2 on cultured human DCs. We show that PGE2 in the absence of LPS stimulation is capable of inducing the production of IL-12 by DCs. Although PGE2 on its own was a weak stimulus of IL-12 production, PGE2 in concert with TNF-α induced the production of high levels of IL-12 in DC cultures. Moreover, the combination of PGE2 and TNF-α turned out to be a potent stimulus of DC maturation.

Materials and Methods

Media and Reagents. The medium used in this study was RPMI 1640 supplemented with 1% heat-inactivated (30 min, 56°C) pooled human AB serum, 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM l-glutamine, 10 mM Hepes, 0.1 mM nonessential amino acids, 1 mM pyruvate, and 5×10^{-5} M 2-mercaptoethanol (all from Boehringer Ingelheim Bioproducts, Vienna, Austria). Human albumin (for intravenous use; Octapharma, Vienna, Austria) was added to a final concentration of 2 mg/ml (= complete medium). Recombinant human GM-CSF (Leucomax; 1.11×10^7 U/mg) was from Novartis (Basel, Switzerland). Recombinant human IL-4 (2×10^7 U/mg) was supplied by the Schering-Plough Research Institute (Kenilworth, NJ). Recombinant human TNF-α (10^7 U/mg) was purchased from Genzyme (Cambridge, MA). PGE2 was purchased from Sigma Chemical Company (St. Louis, MO) and from Calbiochem-Novabiochem International (San Diego, CA). Forskolin, *N*-2-*O*-dibutyryl-cAMP (db-cAMP), LPS (from *Salmonella abortus equi*), and fluorescein-ated Dextran (FITC-DX) were all from Sigma Chemical Co.

Culture of Human DCs. DCs were generated from PBMCs as described (16, 17). In brief, PBMCs were isolated from leukocyte-enriched buffy coats by standard density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), resuspended in complete medium and 5.0×10^7 PBMCs were allowed to adhere in 75-cm^2 cell culture flasks (45 min 37°C). Nonadherent cells were removed and adherent cells were cultured in 10 ml of complete medium containing 1,000 U/ml of each GM-CSF and IL-4. DCs were developed under essentially endotoxin-free conditions as indicated by the absence of spontaneous TNF-α production (<5 pg/ml of TNF-α/5×10^5 DC). On day 2, 5 ml of fresh medium containing 1,000 U/ml of GM-CSF and IL-4 were added. After 5 d of culture, cells were harvested, washed extensively, and recultured in cytokine-containing medium at 3×10^5 cells/ml with or without inflammatory mediators. After 48 h, supernatants were harvested for IL-12 ELISA assays and the cells were analyzed for surface Ag expression by flow cytometry.

Flow Cytometric Measurement of Surface Ag Expression and Endocytic Activity. To determine surface Ag expression, cells (10^5 DC in 50 μl) were labeled with primary mAb in complete medium followed by FITC-conjugated F(ab′)$_2$ fragments of goat anti–mouse Ig (Dako, Glostrup, Denmark). The following mAbs were used: VIM-13 (IgM, anti-CD14, a gift of Dr. W. Knapp, Vienna, Austria), G46-2.6 (IgG1, anti-HLA-ABC), L243 (IgG2a, anti-HLA-DR), HB-15a (IgG2b, anti-CD83), 84H10 (IgG1, anti-CD54), AIDC58 (IgG2a, anti-CD58), BB1 (IgM, anti-CD80), BU63 (IgG1, anti-CD86), 5C3 (IgG1, anti-CD40), 1G10 (IgG1, anti-CD43), G44-26 (IgG2b, anti-CD44). Washes were in HBSS containing 0.2% albumin. After the last wash, the cells were stored in HBSS containing 0.2% albumin and 2% formaldehyde.

The endocytic activity of DCs was measured as described previously (12). FITC-DX was used to measure mannose receptor–mediated endocytosis. Cells (10^5) were incubated with FITC-DX (0.5 mg/ml) for 30 min at 37°C (control at 0°C) and then washed extensively. The samples were analyzed on a FACScan® (Becton Dickinson, Mountain View, CA). Data were analyzed and presented using CellQuest® software from Becton Dickinson.

Quantitation of IL-12. Day-5 DCs (3×10^5 cells/ml) were incubated for 48 h with various stimuli and IL-12 was measured in culture supernatants by ELISA using a commercially available kit (Genzyme) that detects both IL-12 p40 and the bioactive IL-12 p70 heterodimer consisting of p40 and p35. IL-12 was quantitated using a microtiter plate reader.

T Cell Proliferation Assay. DCs were either untreated or treated with TNF-α, PGE2, or PGE2 plus TNF-α for 48 h. Irradiated (3,000 rad) DCs (1×10^3 or 5×10^4) were then cultured with a constant number of 2×10^5 allogeneic T cells in 96-well flat-bottomed tissue culture plates in medium containing 5% pooled human serum. T cell proliferation was measured as [^{3}H]thymidine incorporation (1 μCi/well; 6.7 Ci/mmol, New England Nuclear, Boston, MA). Cells were pulsed during the last 16 h of a 5-d culture period, harvested onto glass fiber filters using a Skatron cell harvester (Skatron Instruments, Lier, Norway), and analyzed in a liquid scintillation counter. Results are expressed as the mean cpm of triplicate wells ± SD.

Results

PGE2 Stimulates IL-12 Production in Human DCs. Day-5 DCs were exposed to graded doses of PGE2, and IL-12 was determined in culture supernatants after 48 h. Fig. 1 *A*

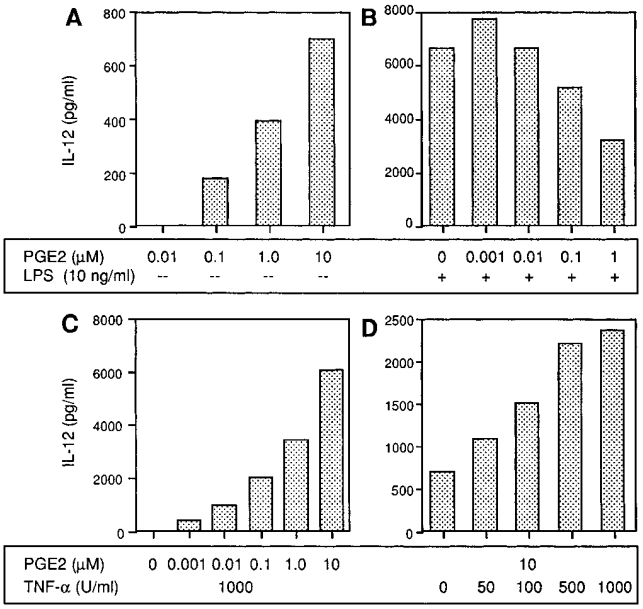

Figure 1. Stimulatory effects of PGE2 on IL-12 production by human DCs. Day-5 DCs were incubated with graded doses of PGE2 either alone (*A*) or in the presence of a constant dose of LPS (*B*) or TNF-α (*C*). Conversely, DCs were also incubated with graded doses of TNF-α in the presence of a constant dose of PGE2 (*D*). After 48 h of culture, supernatants were harvested and IL-12 levels were determined using a specific ELISA. Dose-dependent responses from two out of four independent experiments are shown.

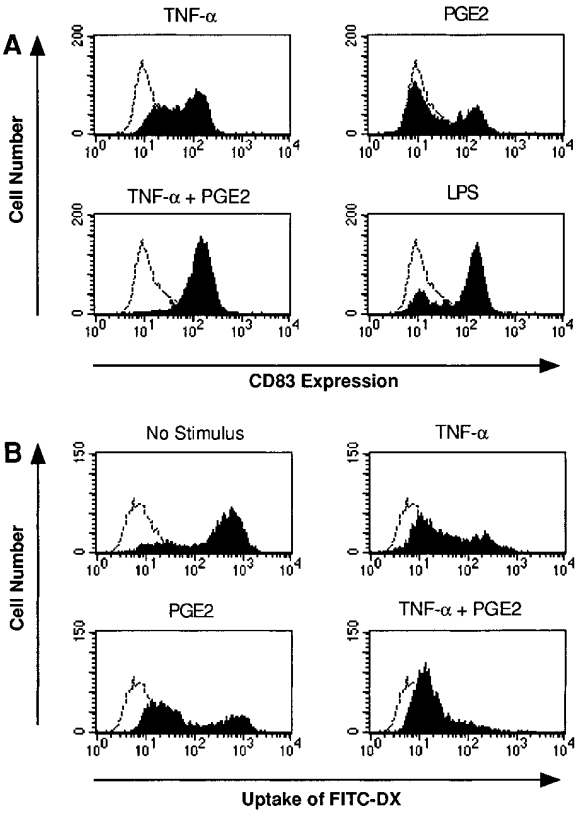

Figure 2. Regulatory effects of PGE2 on CD83 expression and Ag uptake. (*A*) Day-5 DCs were incubated with PGE2 (1 μM), TNF-α (1,000 U/ml), LPS (10 ng/ml), or PGE2 plus TNF-α. After 48 h, cells were harvested and CD83 expression was measured by flow cytometry. The isotype control (IgG2b) is also presented (*dotted lines*). (*B*) Day-5 DCs were incubated with PGE2 (1 μM), TNF-α (1,000 U/ml), or PGE2 plus TNF-α. After 24 h, cells were harvested and incubated with FITC-DX for 30 min at 37°C (controls at 0°C, *dotted lines*), washed, and analyzed by flow cytometry.

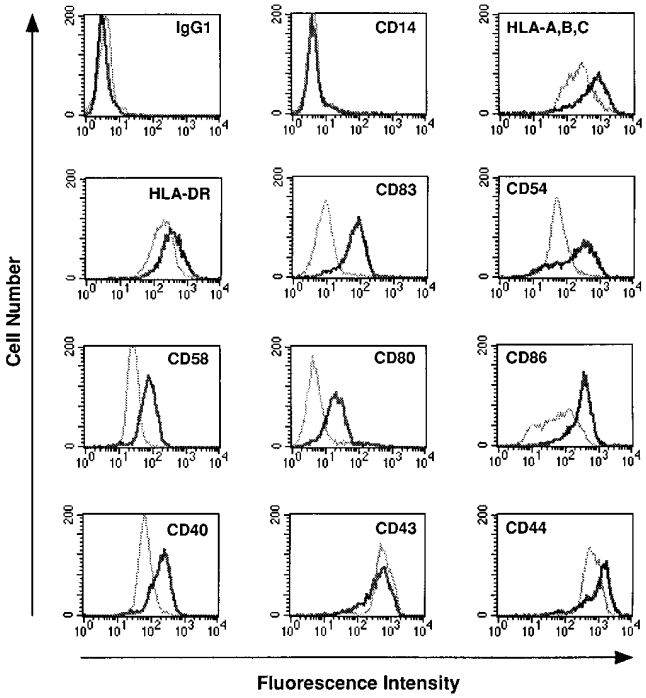

Figure 3. Phenotypic changes of DCs cultured with PGE2 and TNF-α. Day-5 DCs were recultured in the absence (*dotted lines*) or presence of PGE2 (1 μM) plus TNF-α (1,000 U/ml) (*bold lines*) for 48 h and analyzed by flow cytometry for the surface expression of the indicated Ag using the antibodies listed in Materials and Methods.

demonstrates that PGE2 stimulated IL-12 production in a dose-dependent fashion. However, the total amount of IL-12 induced by PGE2 was relatively low. PGE2-dependent IL-12 production by DCs occurred in the absence of endogenous TNF-α production (data not shown). TNF-α has been described to enhance IL-12 production in the context of LPS stimulation (18). Therefore, we tested the effects of combinations of PGE2 and TNF-α on IL-12 production by DCs. In the presence of TNF-α, PGE2 dose dependently stimulated high level IL-12 production in DCs (Fig. 1 *C*). Conversely, the level of IL-12 production also depended on the dose of TNF-α (Fig. 1 *D*). The effects of PGE2 and TNF-α were synergistic since TNF-α alone completely failed to induce IL-12 production by DCs (Fig. 1 *C*). In contrast, PGE2 inhibited the LPS-induced IL-12 production by DCs (Fig. 1 *B*) confirming previous observations (8). PGE2 from two different commercial sources were used with consistent results.

PGE2 and TNF-α Cooperate to Activate Human DCs. IL-12 production by DCs is always associated with DC activation which can be induced by treatment with LPS (14), BCG (13), a monocyte-conditioned medium (19, 20), or by cross-linking of the CD40 Ag (21). DC activation, which has also been referred to as DC maturation, is characterized by the neo-expression of the CD83 Ag (22), by the upregulation of molecules related to Ag presentation (12, 22), and by the downmodulation of endocytic activity (12, 23). Therefore, we then tested the effects of PGE2 and TNF-α on the process of DC maturation.

The combination of PGE2 and TNF-α turned out to be potent in inducing CD83 expression in DCs (Fig. 2 *A*). Addition of PGE2 (1 μM) along with TNF-α (1,000 U/ml) induced CD83 expression in 75% of the cells within 24 h (data not shown) and in almost all cells within 48 h (Fig. 2 *A*). Addition of either substance alone also induced CD83 expression, although to a smaller extent (Fig. 2 *A*). Moreover, treatment with PGE2 plus TNF-α efficiently upregulated the expression of MHC class I and II molecules, adhesion molecules (CD54, CD58), and costimulatory molecules (CD80, CD86) in DCs (Fig. 3). The expression of CD40 and CD44 was also enhanced. Inspection of these cells by phase contrast microscopy revealed a pronounced dendritic morphology with numerous large veils, a feature of mature DCs (data not shown; reference 10). Conversely, the ability to capture soluble Ag (DX) by mannose receptor–mediated endocytosis was almost completely downregulated in DCs cultured in the presence of PGE2 and TNF-α (Fig. 2 *B*). Again, either substance alone also downmodulated Ag uptake, although less efficiently.

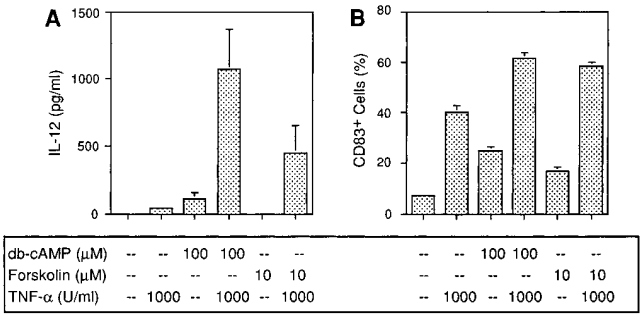

Figure 4. Mimicry of PGE2 effects on IL-12 synthesis and CD83 expression by nonphysiologic modulators of cAMP. Day-5 DCs were incubated with db-cAMP (100 μM) or forskolin (10 μM) either alone or in combination with TNF-α (1,000 U/ml). After 48 h, supernatants were analyzed for the presence of IL-12 using a specific ELISA (*A*) and the cells were analyzed for CD83 expression by flow cytometry (*B*). Data are given as mean ± SEM of four independent experiments.

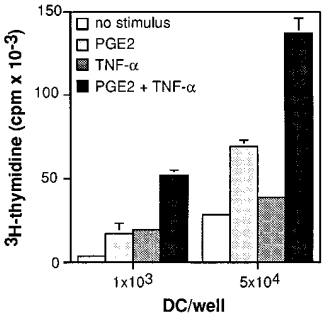

Figure 5. T cell stimulatory capacity of DCs cultured with PGE2. Day-5 DCs were recultured in the absence or presence of PGE2 (10 μM), TNF-α (1,000 U/ml), or PGE2 plus TNF-α. After 48 h the cells were washed, irradiated, and DCs were used as stimulators of allogeneic T cell proliferation. Proliferation was monitored by measuring [methyl-^{3}H]thymidine uptake on day 5 of coculture. Each value represents the mean cpm of triplicate cultures with SD.

PGE2 Effects on DCs Are Mimicked by Nonphysiologic Modulators of cAMP. Most of the effects of PGE2 are mediated by the intracellular second messenger cAMP (24). Therefore, PGE2 effects can be mimicked by nonphysiologic modulators of cAMP such as forskolin and db-cAMP. To investigate the mechanism of the stimulatory effects of PGE2 on DCs, we tested the effects of forskolin or db-cAMP either alone or in the presence of TNF-α on IL-12 synthesis and CD83 expression in human DCs. Fig. 4 *A* demonstrates that db-cAMP (100 μM) stimulated low level IL-12 production and synergized with TNF-α to induce substantial IL-12 production by DCs. CD83 expression was also induced by db-cAMP (Fig. 4 *B*). Forskolin at 10 μM failed to induce IL-12 production (Fig. 4 *A*). Higher concentrations of forskolin could not be tested due to the high toxicity of this compound. However, in the presence of TNF-α forskolin at 10 μM induced measurable IL-12 production (Fig. 4 *A*). Forskolin also induced CD83 expression (Fig. 4 *B*).

PGE2 Enhances the Accessory Cell Capacity of Human DCs. High levels of adhesion molecules expressed by mature DCs (Fig. 3) contribute to the T cell stimulatory potential of DCs by facilitating the formation of clusters with T cells (11). DCs treated with PGE2 and TNF-α were most potent in cluster formation with allogeneic T cells (data not shown). Either substance alone also enhanced cluster formation, although at a lower level.

The formation of clusters serves to establish close contacts between DCs and T cells, thereby favoring TCR engagement and T cell activation (11). Fig. 5 shows that DCs treated with PGE2 and TNF-α also exhibited the highest capacity to stimulate allogeneic T cell proliferation. Treatment of DCs with either substance alone also enhanced T cell proliferation, but to a smaller extent (Fig. 5).

Discussion

PGE2 has been demonstrated to be a potent inhibitor of the LPS-induced human IL-12 production (8). In the present work, we demonstrate that PGE2 in the absence of LPS is capable of stimulating IL-12 production by human DCs. PGE2 per se was a weak stimulus (Fig. 1 *A*), but was synergized with TNF-α to induce the production of high levels of IL-12 by DC (Fig. 1, *C* and *D*). This is consistent with previous reports that demonstrated that PGs are relatively weak inflammatory stimuli, but potentiate inflammatory reactions in the presence of other mediators (6, 7). Moreover, we show that PGE2 contributes to DC maturation (11) which is characterized by the downmodulation of mannose receptor–mediated endocytosis (Fig. 2 *B*), by the upregulation of molecules that are important for T cell activation (Fig. 3), as well as by the neo-expression of the CD83 Ag (Fig. 2 *A*). DC maturation can also be induced by monocyte-conditioned medium (19, 20). Our findings suggest that PGE2, which is released by activated monocytes, contributes to the DC maturation function of monocyte-conditioned medium. PGE2-treated DCs also exhibited an increased accessory cell potential which was evident as an enhanced capacity to form clusters with allogeneic T cells (data not shown) and to stimulate allogeneic T cell proliferation (Fig. 5).

Our data suggest the involvement of a cAMP signaling pathway in DC maturation since addition of db-cAMP and forskolin, which are known to increase intracellular levels of cAMP (24), mimicked the effects of PGE2. Similarly, in human monocytes, cAMP synergized with TNF-α to upregulate the synthesis of IL-1β (25), suggesting that the TNF-α signal transduction pathway that leads to intracellular accumulation of ceramide (26) and the PG pathway via elevation of the intracellular second messenger cAMP (24), cooperate to induce cytokine production by APCs (reference 25 and this work).

DCs obviously produce IL-12 at different anatomical sites and different states of their development. DCs residing in peripheral tissues are immature. Tissue DCs are actively endocytosing Ag. DCs cultured with GM-CSF and IL-4 exhibit high endocytic capacity and appear to correspond to these tissue DCs (11). At this state, PGE2 has stimulatory effects and strongly enhances IL-12 synthesis in the presence of TNF-α (Fig. 1, *C* and *D*). The capability of immature DCs to produce large amounts of IL-12 in response to inflammatory conditions reflects the contribution of DCs to

innate resistance and primarily functions to activate NK cells (27). Accumulation of inflammatory mediators then prompts DCs to migrate to the T cell areas of secondary lymphoid organs where they present Ags to recirculating T cells. During contact with Ag-specific T cells, IL-12 produced by DCs after triggering of their CD40 antigen by T cell CD40 ligand (28, 29) favors the generation of Th1 type T cell responses. At this level, PGE2 appears to inhibit T cell activation and to selectively suppress the production of Th1 cytokines (30–32). Taken together, PGE2 appears to exert both stimulatory and inhibitory effects on the development of Th1 type T cell responses depending on the site of PGE2 action.

The combination of PGE2 and TNF-α induced full DC activation (Figs. 2 and 3), which included IL-12 production by DCs (Fig. 1). This mechanism of activation, which is independent of infectious Ags, may play a role in autoimmune diseases. Mononuclear cells derived from inflamed rheumatoid arthritis joints exhibited enhanced production of both PG (33) and TNF-α (34), which can cooperate to increase IL-12 production (Fig. 1). IL-12 production is indeed increased in synovial mononuclear cells from arthritis patients (35) and in tubular epithelial cells of mice with lupus nephritis (36). Moreover, IL-12 is known to accelerate the development of autoimmune diseases (37). Furthermore, the induction of DC maturation by PGE2 and TNF-α in the inflammatory lesions may result in continuous presentation of self Ag by fully activated DCs in vivo, and may thereby support the development of autoimmunity.

In conclusion, our data indicate that PGE2 must also be considered an important activator of human DCs. The beneficial effect of nonsteroidal antiinflammatory drugs in inflammatory diseases such as rheumatoid arthritis may relate to the inhibition of PGE2-mediated DC activation.

We gratefully acknowledge the excellent technical assistance of Christine Papesh. The authors are grateful to Dr. Nikolaus Romani for careful reading of the manuscript and to Dr. G. Utermann for kind support.

This work was supported by grant P11758MED of the Austrian Science Fund (to Martin Thurnher).

Address correspondence to Dr. Martin Thurnher, The Department of Urology, Anichstrasse 35, 6020 Innsbruck, Austria. FAX: 43-512-504-4817; E-mail: martin.thurnher@uibk.ac.at

Received for publication 21 July 1997 and in revised form 2 September 1997.

References

1. Fearon, D.T., and R.M. Locksley. 1996. The instructive role of innate immunity in the acquired immune response. *Science (Wash. DC).* 272:50–53.
2. Portanova, J.P., Y. Zhang, G.D. Anderson, S.D. Hauser, J.L. Masferrer, K. Seibert, S.A. Gregory, and P.C. Isakson. 1996. Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. *J. Exp. Med.* 184:883–891.
3. Garrison, J.C. 1990. Histamin, bradykinin, 5-hydroxytryptamine and their antagonists. *In* The Pharmacological Basis of Therapeutics. A. Gilman, T.W. Rall, A.S. Nies, and P. Taylor, editors. Pergamon Press, New York. 574–599.
4. Moncada, S., R.M. Palmer, and E.A. Higgs. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109–142.
5. Vane, J.R., and R.M. Botting. 1994. Biological properties of cyclooxygenase products. *In* The Handbook of Immunopharmacology. F.M. Cunningham, editor. Academic Press, London. 61–97.
6. Williams, T.J., and J. Morley. 1973. Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature (Lond.).* 246:215–217.
7. Moncada, S., S.H. Ferreira, and J.R. Vane. 1973. Prostaglandins, aspirin-like drugs and the oedema of inflammation. *Nature (Lond.).* 246:217–219.
8. van der Pouw Kraan, T.C., L.C. Boeije, R.J. Smeenk, J. Wijdenes, and L.A. Aarden. 1995. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J. Exp. Med.* 181:775–779.
9. Strassmann, G., V. Patil Koota, F. Finkelman, M. Fong, and T. Kambayashi. 1994. Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2. *J. Exp. Med.* 180:2365–2370.
10. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9:271–296.
11. Cella, M., F. Sallusto, and A. Lanzavecchia. 1997. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol.* 9:10–16.
12. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med.* 182:389–400.
13. Thurnher, M., R. Ramoner, G. Gastl, C. Radmayr, G. Böck, M. Herold, H. Klocker, and G. Bartsch. 1997. Bacillus Calmette-Guérin mycobacteria stimulate human blood dendritic cells. *Int. J. Cancer.* 70:128–134.
14. Verhasselt, V., C. Buelens, F. Willems, D. DeGroote, N. Haeffner-Cavaillon, and M. Goldman. 1997. Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells – evidence for a soluble CD14-dependent pathway. *J. Immunol.* 158:2919–2925.
15. Austyn, J.M. 1996. New insights into the mobilization and phagocytic activity of dendritic cells. *J. Exp. Med.* 183:1287–1292.
16. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation

of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J. Exp. Med.* 179:1109–1118.
17. Romani, N., S. Gruner, D. Brang, E. Kämpgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P.O. Fritsch, R.M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* 180:83–93.
18. DeKruyff, R.H., R.S. Gieni, and D.T. Umetsu. 1997. Antigen-driven but not lipopolysaccharide-driven IL-12 production in macrophages requires triggering of CD40. *J. Immunol.* 158:359–366.
19. Romani, N., D. Reider, M. Heuer, S. Ebner, E. Kämpgen, B. Eibl, D. Niederwieser, and G. Schuler. 1996. Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. *J. Immunol. Methods.* 196:137–151.
20. Bender, A., M. Sapp, G. Schuler, R.M. Steinman, and N. Bhardwaj. 1996. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J. Immunol. Methods.* 196:121–135.
21. Caux, C., C. Massacrier, B. Vanbervliet, B. Dubois, C. Van Kooten, I. Durand, and J. Banchereau. 1994. Activation of human dendritic cells through CD40 cross-linking. *J. Exp. Med.* 180:1263–1272.
22. Zhou, L.J., and T.F. Tedder. 1996. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. *Proc. Natl. Acad. Sci. USA.* 93:2588–2592.
23. Thurnher, M., C. Papesh, R. Ramoner, G. Gastl, G. Böck, C. Radmayr, H. Klocker, and G. Bartsch. 1997. In vitro generation of CD83+ human blood dendritic cells for the active tumor immunotherapy. *Exp. Hematol. (NY).* 25:232–237.
24. Phipps, R.P., S.H. Stein, and R.L. Roper. 1991. A new view of prostaglandin E regulation of the immune response. *Immunol. Today.* 12:349–352.
25. Lorenz, J.J., P.J. Furdon, J.D. Taylor, M.W. Verghese, G. Chandra, T.A. Kost, S.A. Haneline, L.A. Roner, and J.G. Gray. 1995. A cyclic adenosine 3′,5′-monophosphate signal is required for the induction of IL-1 beta by TNF-α in human monocytes. *J. Immunol.* 155:836–844.
26. Sallusto, F., C. Nicolo, R. De Maria, S. Corinti, and R. Testi. 1996. Ceramide inhibits antigen uptake and presentation by dendritic cells. *J. Exp. Med.* 184:2411–2416.
27. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251–276.
28. Cella, M., D. Scheidegger, K. Palmer Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. 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.* 184:747–752.
29. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kämpgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med.* 184:741–746.
30. Snijdewint, F.G., P. Kalinski, E.A. Wierenga, J.D. Bos, and M.L. Kapsenberg. 1993. Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J. Immunol.* 150:5321–5329.
31. Katamura, K., N. Shintaku, Y. Yamauchi, T. Fukui, Y. Ohshima, M. Mayumi, and K. Furusho. 1995. Prostaglandin E2 at priming of naive CD4+ T cells inhibits acquisition of ability to produce IFN-γ and IL-2, but not IL-4 and IL-5. *J. Immunol.* 155:4604–4612.
32. Hilkens, C.M., A. Snijders, H. Vermeulen, P.H. van der Meide, E.A. Wierenga, and M.L. Kapsenberg. 1996. Accessory cell–derived IL-12 and prostaglandin E2 determine the IFN-γ level of activated human CD4+ T cells. *J. Immunol.* 156:1722–1727.
33. Seitz, M., and W. Hunstein. 1985. Enhanced prostanoid release from monocytes of patients with rheumatoid arthritis and active systemic lupus erythematosus. *Ann. Rheum. Dis.* 44:438–445.
34. Firestein, G.S., J.M. Alvaro Gracia, R. Maki, and J.M. Alvaro Garcia. 1990. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. *J. Immunol.* 144:3347–3353.
35. Schlaak, J.F., I. Pfers, K.H. Meyer Zum Büschenfelde, and E. Marker Hermann. 1996. Different cytokine profiles in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and seronegative spondylarthropathies. *Clin. Exp. Rheumatol.* 14:155–162.
36. Fan, X., B. Oertli, and R.P. Wuthrich. 1997. Up-regulation of tubular epithelial interleukin-12 in autoimmune MRL-Fas(lpr) mice with renal injury. *Kidney Int.* 51:79–86.
37. Huang, F.P., G.J. Feng, G. Lindop, D.I. Stott, and F.Y. Liew. 1996. The role of interleukin 12 and nitric oxide in the development of spontaneous autoimmune disease in MRLMP-*lpr/lpr* mice. *J. Exp. Med.* 183:1447–1459.