Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 331 (2005) 187–193
BBRC
www.elsevier.com/locate/ybbrc

PGE/cAMP and GM-CSF synergise to induce a pro-tolerance cytokine profile in monocytic cell lines

Vivien Grant, Anne E. King, Elena Faccenda, Rodney W. Kelly *

Medical Research Council, Human Reproductive Sciences Unit, University of Edinburgh Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK

Received 16 March 2005
Available online 30 March 2005

Abstract

This study demonstrates a synergistic action of prostaglandin E and GM-CSF which causes the release of pro-tolerant cytokines in two monocyte cell lines: U937 and ML-1. The prostaglandin effect is cyclic AMP dependent since stimulators of adenyl cyclase such as forskolin (fsk) can replace PGE. Fsk and GM-CSF combinations raised messenger RNA for IL-10, interleukin-1 receptor antagonist (IL-1ra), and CD14 as well as the released proteins. Effective levels of interleukin 12 are reduced. In these respects, the monocyte cells resemble the alternatively activated or tumour associated macrophages. A differential pattern in co-stimulatory molecule expression is seen; CD80 is unchanged but CD86 is markedly elevated and such a change is not seen in the alternatively activated macrophage but has been previously reported in monocytes resident in the non-inflamed gut. Control of leukocyte responses by two agents acting in synergy could be effective in critical situations such as discrimination between pathogens and commensal bacteria, etc. Monocytes modified in such a way could provide a pro-tolerant environment (high IL-10, low IL-12) for antigen presentation by dendritic cells and thus may contribute to a normally permissive milieu, e.g., for food absorption.

Keywords: Prostaglandin E; GM-CSF; Monocytes; IL-10; Immunological tolerance; U937; ML-1; Autoimmune disease

Prostaglandins are commonly regarded as pro-inflammatory agents, particularly since non-steroidal anti-inflammatory drugs (NSAID) have a main function of inhibiting prostaglandin biosynthesis. However, such inflammatory effects are likely to be mediated at the level of the vascular endothelium and modulate leukocyte ingress into tissue by a vaso-active mechanism which relies on a synergism between PGE and bradykinin [1] or chemokines such as CCL8 [2,3]. In contrast, there are important immunomodulatory roles of PGE which can suppress a cellular immune response. Direct actions of PGE on leukocytes are mainly suppressive: free-oxygen radical production by the neutrophil [4], T cell replication [5], monocyte sensitivity [6], and phagocytosis [7] are all reduced by cAMP-dependent mechanisms. In addition, cell-mediated immune responses are dampened after burn injury by a cyclooxygenase-2 (the inducible key synthetic enzyme)-dependent mechanism [8].

However, not only suppression but also immunological tolerance is dependent on PGE [9–11] and can be prevented or broken by non-steroidal anti-inflammatory drugs [12,13]. PGE has been implicated in oral tolerance in experiments in mice where a transgenic T cell receptor was engineered to recognise hen-lysozyme. Such animals, when fed the antigen, did not exhibit pathology but when a COX-2 inhibitor was also administered they suffered serious inflammation [14]. The tolerance and suppression of inflammatory response was attributed to PGE from cells of the *lamina propria* of the gut. PGE can also affect the maturation of dendritic cells with cells exposed to PGE releasing lower levels of

* Corresponding author.
E-mail address: r.kelly@hrsu.mrc.ac.uk (R.W. Kelly).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.137

IL-12 and resulting T cells releasing T-helper 2 type cytokines [15].

PGE and elevated cAMP levels are known to act on LPS stimulated macrophages by switching two key cytokines involved in immunological tolerance: IL-10 is stimulated [16] and IL-12 [17] is reduced. Curiously these effects are LPS dependent which might suggest that other agents, released by LPS, may be involved.

IL-12 can counteract tolerance induction [18–20] and may play a significant role in induction of autoimmunity [21]. In contrast, IL-10 appears to be a major contributor to immunological tolerance [22,23] and plays a key role in oral tolerance and regulatory T cell formation and function [24,25]. One mechanism by which tolerant T cells are produced is by an action of IL-10 on the maturing dendritic cell [26] and therefore IL-10 secretion by neighbouring cells will affect the outcome of antigen presentation. In this respect, the monocyte, which potentially secretes both IL-10 and IL-12, may play an accessory, but nonetheless important, role in dendritic cell programming and thus tolerance induction. In support of this, macrophages found at sites where tolerance is encountered, such as those associated with the gut and with cancers, have a distinct phenotype. These cells are referred to as alternately activated, tumour associated or M2 macrophages [27].

Monocyte/macrophage development is also dependent on cytokine environment. In the presence of interferon γ and lipopolysaccharide (LPS), macrophages actively phagocytose pathogen-derived material and can present antigen as complexes on MHC-II structures. However, tumour associated macrophages secrete IL-10 and IL-1ra as well as the chemokine CCL18 and CCL16 [27]. Such macrophages are derived from circulating monocytic precursors [28] which may be induced into tissue by chemotactic agents such as CCL2 produced by tumour cells [29]. Although the alternatively activated macrophage is likely to further a tolerant environment, the agents responsible for inducing this phenotype are uncertain although IL-4, IL-10, and IL-13 are all implicated.

Although PGE alters monocyte cytokine release, its role in monocyte differentiation is unknown. Here we have used two committed-progenitor, monocytic cell lines, U937 and ML-1, to study the role of PGE together with GM-CSF in changing monocyte cytokine release and found an unexpected synergism.

Experimental

Cell culture

Both U937 and ML-1 cells were grown in Standard culture medium (Dutch Modification RPMI 1640 (PAA Laboratories, Somerset) with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamycin (20 μg/ml) (Sigma, Poole) also plasmocin (5 μg/ml) (Autogen-Bioclear)). Cells were routinely grown in a low plasmocin concentration to maintain mycoplasma-free status. Lines were rigorously tested for mycoplasma using a nested PCR based technique (ATCC, Manassas, Virginia). Cells were treated in Standard culture medium modified to 2% FCS. Fsk (Sigma, Poole) was used at 50 μM and Rolipram (Sigma, Poole) at 1 μM. GM-CSF (Peprotech., London) was used at 5 ng/ml, except in the dose–response studies. PGE in ethanol was added to give a final concentration of 1 μM maintaining ethanol concentrations below 0.01%.

ELISA measurements

Culture supernatants were stored at −20 °C until assayed. Assays used matched antibody pairs (R&D, Abingdon, Oxford) and were conducted according to the manufacturer's protocol.

Performance data. IL-10 assay: standard range 500–7.8 pg/ml, intra-assay variation 4.9%, inter-assay variation 6.6%. Soluble CD14 assay: standard range 16,000–250 pg/ml, intra-assay variation = 4.8%, inter-assay = 6.7%. TNFα assay: standard range 2000–31.2 pg/ml, intra-assay variation = 3.83%, inter-assay = 5.02%. cAMP assay: standard range 200–0.78 pmol/ml, intra-assay variation = 2.5%, inter-assay = 11.2%. IL-1 receptor antagonist assay: standard range 10,000–78 pg/ml, intra-assay variation = 5.2%, inter-assay variation = 9.8%.

RNA isolation and real-time RT-PCR quantification

Cell lines were plated out in 6-well plates at a concentration of 4×10^5 cells/ml and 4 ml/well. Cells were stimulated with PGE and GM-CSF, with fsk and GM-CSF or with rolipram, fsk, and GM-CSF. Following incubation cells were spun down, supernatant was collected, and RNA was extracted with Tri-Reagent (Molecular Research Center, Cincinnati, OH) as per manufacturer's protocol. RNA separation was effected with Phaseloc gel tubes (Eppendorf, Hamburg, Germany). cDNA was prepared from RNA using reverse transcriptase and random hexamers (Applied Biosystems, Foster City, CA). Variability of the reverse transcriptase reaction was determined by eight separate reverse transcriptase reactions using the same RNA sample, which gave a relative standard deviation of 1.9%.

cDNA templates were amplified with a Taqman 7700 (Applied Biosystems, Foster City, CA) using FAM/TAMRA dyes for the probes. Ribosomal (18S) RNA was amplified using a VIC/TAMRA probe (Applied Biosystems) and used as an internal control. DNA amplification was performed with hot-start *Taq* and with the standard Taqman protocol of 2 min at 50 °C, 10 min at 90 °C followed by 40 cycles of 95 °C denaturing step (15 s), and 60 °C annealing/extension (1 min). The fluorescent signals are recorded during the annealing/extension phase at each cycle. The C_t (related to the cycle number at which signal appears) for the specific RNA (FAM signal) and the 18S were evaluated. The absolute relative quantitation was achieved using the formula $2^{-\Delta\Delta C_t}$, which relates the amount of cDNA of the specific amplicon to the 18S internal control and the control cDNA.

Primer/probe combinations were designed with Primer Express software (Applied Biosystems). Sequences were validated by BLAST searches to show that the amplified sequence was unique and linearity of response was checked by amplifying serially diluted cDNA samples and plotting the corrected values against dilution. Sequences used were:

CD80, forward 5′-TCCACGTGACCAAGGAAGTG-3′, reverse 5′-CCAGCTCTTCAACAGAAACATTGT-3′, Probe 5′-AAGAAGTGGCAACGCTGTCCTGTGG-3′
CD86, forward 5′-CAGACCTGCCATGCCAATT-3′, reverse 5′-TTCCTGGTCCTGCCAAAATACTA-3′, Probe 5′-CAAACTCTCAAAACCAAAGCCTGAGTGAGC-3′
IL-12 p35, forward 5′-CCACTCCAGACCCAGGAATG-3′, reverse 5′-TGTCTGGCCTTCTGGAGCAT-3′, Probe 5′-TCCCATGCCTTCACCACTCCCAA-3′

IL-12p40, forward 5′-CGGTCATCTGCCGCAAA-3′, reverse 5′-TGCCCATTCGCTCCAAGA-3′, Probe 5′-CCAGCATTAGCGTGCGGGCC-3′
IL-10, forward 5′-CTACGGCGCTGTCATCGAT-3′, reverse 5′-TGGAGCTTATTAAAGGCATTCTTCA-3′, Probe 5′-CTTCCCTGTGAAAACAAGAGCAAGGCC-3′
IL-1rec. antagonist, forward 5′-TTGCAAGGACCAAATGTCAATT-3′, reverse 5′-CCATGGATTCCCAAGAACAGA-3′, Probe 5′-CATGAGGCTCAATGGGTACCACATCTATCTTTT-3′
CD14, forward 5′-GCGCTCCGAGATGCATGT-3′, reverse 5′-AGCCCAGCGAACGACAGA-3′, Probe 5′-TCCAGCGCCCTGAACTCCCTCA-3′
CD88, forward 5′-GGCGGTAGCCGACTTCCT, reverse 5′-GCTGTACAATGGACGTGAACAAG-3′, Probe 5′-TCCTGCCTGGCGCTGCCC-3′.

FACS analysis

Cultured cells were harvested, centrifuged, and resuspended in FACS buffer (phosphate-buffered saline (Sigma), 1% fetal calf serum (Sigma, Poole, UK) and 1 mg/ml sodium azide) at $\approx 5 \times 10^5$ cells in 100 μl. Ten microliters of antibody was added to each tube, mixed, and incubated on ice in the dark for 30 min. Each tube was washed three times in FACS buffer; on final wash cells were suspended in ≈500 μl FACS buffer with 0.5% *p*-formaldehyde. Samples were analysed with a Coulter EPICS XL Flow Cytometer (Beckman-Coulter). Antibodies used were: CD14-FITC (Serotech Oxford, UK) (MCA596F); CD80-FITC Immunotech (IM1853); CD86-PE (Beckman-Coulter) (IM2729). Isotype controls: mouse IgG2akappa-FITC Sigma (F6522); mouse IgG1kappa-FITC Sigma (F6397); mouse IgG2bkappa-PE Sigma (P4935).

Statistical analysis

Significant difference was determined using Kruskal–Wallis non-parametric analysis of variance (Instat) together with a Dunn's multiple comparisons test to assign individual significant differences.

Results

Synergistic effect of forskolin and GM-CSF

The synergistic effect of fsk and GM-CSF is more potent than PGE and GM-CSF (possibly due to instability or catabolism of PGE) and was used to demonstrate cytokine changes in both U937 and ML-1 cells (Fig. 1A). mRNA for IL-10 was increased more than 1000-fold in both cell lines. This rise in mRNA is accompanied by a 30-fold increase in IL-10 protein released and measured in the culture medium after 48 h. Similar synergistic increases are also seen for IL-1 receptor antagonist (Fig. 1A). mRNA for CD86 is increased around 100-fold in both U937 and ML-1 cells but there is no increase seen in CD80. This reflects a differential expression of CD86 over CD80 which was confirmed by fluorescence activated cell analysis in the presence of rolipram (a type IV phosphodiesterase inhibitor) which accentuates the cAMP effect. No increase in CD80 was observed whilst CD86 showed a marked increase (Fig. 1B).

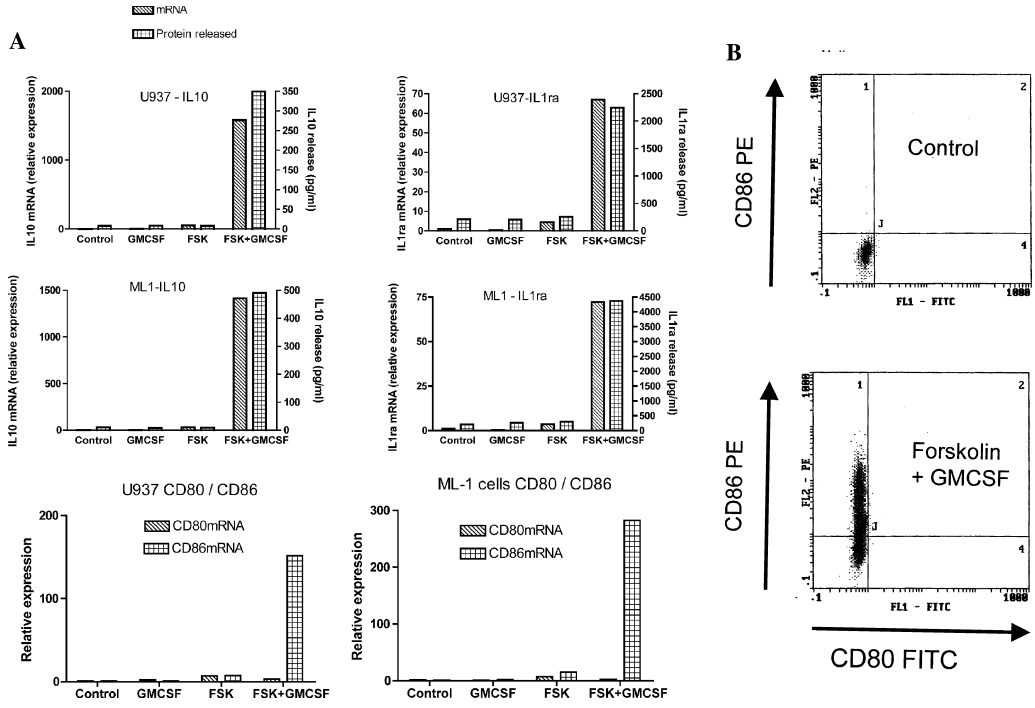

Fig. 1. Synergistic effects of fsk with GM-CSF are evident in both U937 and ML-1 cells. mRNAs for IL-10 and IL-1 receptor antagonist are stimulated 1500- and 70-fold, respectively, in both U937 and ML-1 cells. Released IL-10 and IL-1ra protein are increased synergistically in each cell line (results typical of three experiments). Whereas CD86 (B7.2) (as mRNA or as protein measured by FACS) is increased synergistically by elevated cAMP and GM-CSF, CD80 (B7.1) is not.

Time course and dose–response

Time courses of the increased IL-10 expression showed a small effect at 20 h of fsk and rolipram alone in raising IL-10 but this was synergistically increased by the addition of GM-CSF (Fig. 2). PGE and GM-CSF interact synergistically to stimulate IL-10 expression with a dose–response seen in both PGE and GM-CSF concentrations (Fig. 2B). Prostaglandin concentration above 1 μM showed no added effect and near optimal effects of GM-CSF can be seen at 5 ng/ml.

PGE/GM-CSF effects

The combination of PGE and GM-CSF also stimulates CD14 mRNA and IL-1 receptor antagonist in U937 cells (Fig. 3). Cells were incubated with PGE, GM-CSF, and combinations for 48 h, cells were then washed and exposed to lipopolysaccharide for 6 h before harvesting RNA. Thus, the effect on mRNA was not dependent on the presence of stimulating agents. CD88, the C5a receptor, is also stimulated showing an increased potential responsiveness to complement fragments. No synergistic effects of TGFβ and GM-CSF on IL-10 mRNA were observed (data not shown).

CD14 and IL-12 synthesis

Although CD14 expression was increased by the synergistic treatment of PGE and GM-CSF (Fig. 3), this was accompanied by a large release in soluble CD14 (Fig. 4) and no significant increase in cell surface CD14 as measured by FACS analysis could be observed (data not shown). There is thus the possibility that the increased mRNA for CD14 is predominantly driving soluble CD14 release.

The analysis of the expression of the two components of the hetero-dimeric IL-12 molecule (p35 and p40) shows that p40 expression is increased by PGE + GM-CSF (times 29 ± 19; mean ± SEM, $n = 4$ separate experiments) whereas p35 expression is decreased (times 0.75 ± 0.25; mean ± SEM, $n = 4$ separate experiments). The ratio of p35/p40 was significantly ($p < 0.001$) reduced in a synergistic fashion by PGE with GM-CSF (Fig. 4).

Discussion

We have shown an unexpected effect of GM-CSF synergising with PGE to induce a phenotype which would be pro-tolerant. Although PGE is a physiological agent for raising cAMP through its EP2 and EP4 receptors, the EP2 receptor is downregulated by PGE in U937 cells [30] and PGE is susceptible to catabolism by the 15-prostaglandin dehydrogenase enzyme. Thus, in vitro, treatment with fsk (with or without rolipram) has been used here to attain high cAMP levels. The cytokine profile produced by monocytes matured in this way is similar to that of the alternatively activated macrophage [27] or the tumour associated macrophage. Thus, there is high secretion of IL-10 and IL-1 receptor antagonist. Effects on the two components of the IL-12 hetero-dimer are different with some stimulation of p40 (which is also a component of other cytokines) and a drop in expression of p35. This effect on IL-12 is similar to that seen in the maturing dendritic cell [31]. However, confusion has arisen because of p40 being used as a measure of IL-12 [32]. When measurements of holo-IL-12 are

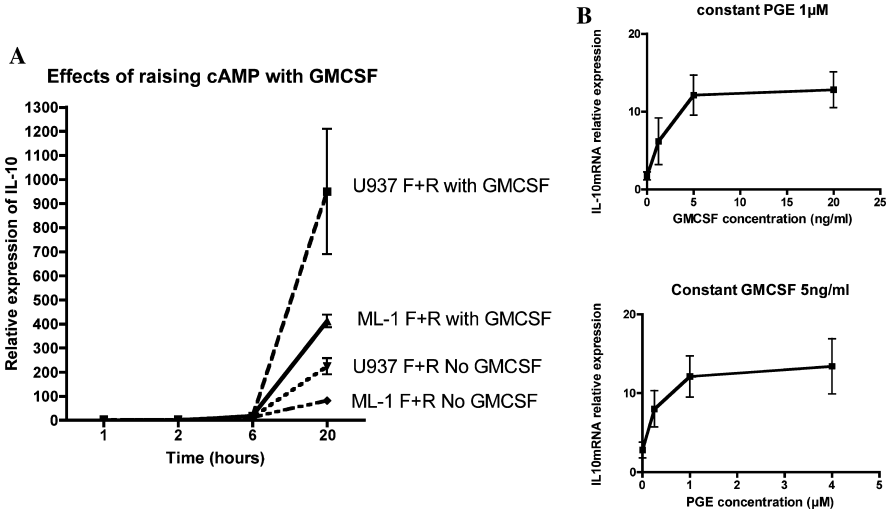

Fig. 2. Time course of increasing IL-10 mRNA as measured by real-time quantitative PCR. (A) At 20 h there is a synergistic effect of GM-CSF with fsk and rolipram. (B) IL-10 mRNA is increased by a combination of GM-CSF and PGE with dose-dependency for both GM-CSF (in the presence of 1 μM PGE) and for PGE in the presence of GM-CSF (5 ng/ml).

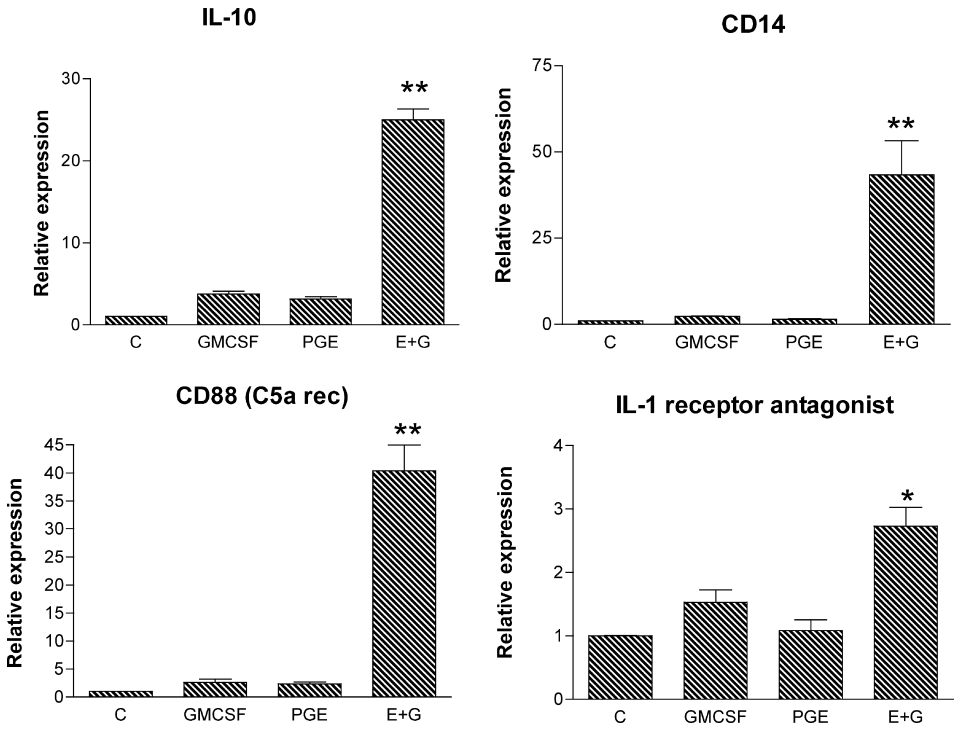

Fig. 3. Combinations of PGE and GM-CSF induce significant increases in IL-10, CD14, CD88, and IL-1 receptor antagonist. Mean ± SEM with a minimum of four experimental replicates for each. *$p < .05$, **$p < .01$.

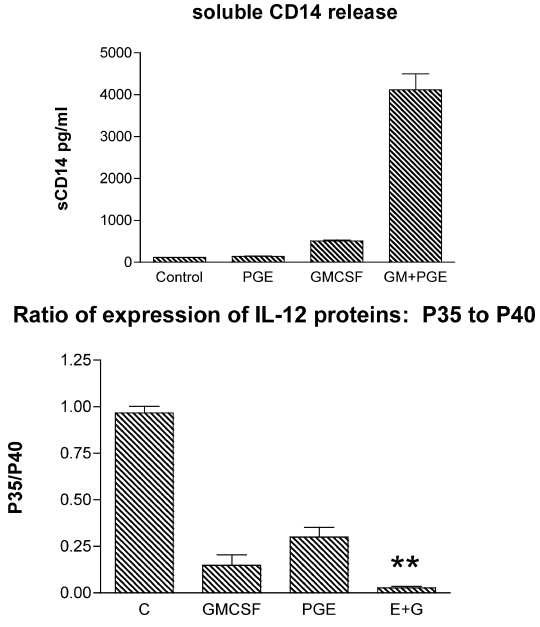

Fig. 4. Upper panel: although there is a large increase in CD14 mRNA, this is reflected by a large increase in soluble protein released from the cells. Lower panel: the combination of PGE and GM-CSF has a differential effect on the expression of the two components of the hetero-dimer IL-12, p35, and p40. The ratio of p35 to p40 is very effectively decreased by this combination (mean ± SEM of four experiments). **$p < .01$.

used, then PGE matured dendritic cell produces considerably less IL-12 (p70) than similar cells matured without PGE [31,33].

A distinguishing feature of the phenotype seen in these studies is the differential effect on co-stimulatory molecule expression. Although the effects of differentially expressed CD80 and CD86 are controversial [34], the stimulation of CD86 but not CD80 (Fig. 1) may reflect the induction of the pro-tolerant state since macrophages in the *lamina propria* of the normal resting gut are reported to be $CD86^+$ $CD80^-$ [35], a pattern that is altered to $CD86^+$, $CD80^+$ in the case of inflammatory bowel disease [35].

The role of GM-CSF in the maturation of cells of the monocytic and granulocyte lineage has been established [36]. However, the effects of GM-CSF on cytokine release from monocytic cells are less clear with the suggestion that IL-1 and/or TNFα are necessary for pro-inflammatory cytokine release [36]. GM-CSF is necessary for the derivation of dendritic cells from monocyte precursors, but later stages of maturation can be affected by PGE (or elevated cAMP) to produce a pro-tolerant dendritic cell [37]. In the absence of PGE and in the presence of stimulatory agents such as LPS, both the dendritic cell and the monocyte will produce Th-1 associated cytokines such as IL-12 and process T cells accordingly, the dendritic cell directly and the monocyte/macrophage mainly by providing the milieu favouring cellular cytotoxicity. Since the monocyte is a major source of PGE [38], experiments depending on the effects of PGE are more readily performed in systems where cells are at a single, early, developmental stage such as the monocytic cell lines U937 and ML-1. In studies on

the maturation of dendritic cells, it has been shown that the monocyte-derived dendritic cell is devoid of PGE [39] which has probably allowed sensitivity to this prostaglandin to be recognised. Both PGE and GM-CSF have pro-inflammatory character. PGE enhances oedema and neutrophil influx, particularly as a synergistic partner with bradykinin [1] and CXCL-8 (IL-8) [2,3,40], and it is likely that these are the inflammatory pathways targeted by the non-steroidal anti-inflammatory drugs [41]. GM-CSF is known to potentiate inflammation induced by lipopolysaccharide, involving increased release of TNFα [42,43]. In contrast, both PGE and GM-CSF also display immunomodulatory actions which could be regarded as anti-inflammatory: PGE inhibits IL-12 [17] and GM-CSF restores innate immune modulators but not pro-inflammatory agents in a dexamethasone suppressed model [44].

This novel action of PGE, working in synergy with GM-CSF on monocytes, would promote a pro-tolerance environment for antigen presentation. The corollary of this is that non-steroidal anti-inflammatory drugs would weaken the normal tolerant state in areas of mucosa exposed to vital food antigens. This would accord with the known effects of such drugs in breaking tolerance [13].

References

[1] T.J. Williams, Prostaglandin E2, prostaglandin I2 and the vascular changes of inflammation, Br. J. Pharmacol. 65 (1979) 517–524.
[2] S.J. Foster, D.M. Aked, J.M. Schroder, E. Christophers, Acute inflammatory effects of a monocyte-derived neutrophil-activating peptide in rabbit skin, Immunology 67 (1989) 181–183.
[3] I.G. Colditz, Effect of exogenous prostaglandin E2 and actinomycin D on plasma leakage induced by neutrophil activating peptide-1/interleukin-8, Immunol. Cell Biol. 68 (1990) 397–403.
[4] J.E. Smolen, H.M. Korchak, G. Weissmann, Increased levels of cyclic adenosine-3′,5′-monophosphate in human polymorphonuclear leukocytes after surface stimulation, J. Clin. Invest. 65 (1980) 1077–1085.
[5] S. Chouaib, R.J. Robb, K. Welte, B. Dupont, Analysis of prostaglandin E2 effects on T lymphocyte activation, J. Clin. Invest. 80 (1987) 333–340.
[6] R.G. Coffey, V.A. Alberts, L.L. Weakland, Prostaglandin dependent desensitization of human monocyte cAMP responses, J. Leukoc. Biol. 48 (1990) 557–564.
[7] D.M. Aronoff, C. Canetti, M. Peters-Golden, Prostaglandin E2 inhibits alveolar macrophage phagocytosis through an E-prostanoid 2 receptor-mediated increase in intracellular cyclic AMP, J. Immunol. 173 (2004) 559–565.
[8] M.G. Schwacha, C.S. Chung, A. Ayala, K.I. Bland, I.H. Chaudry, Cyclooxygenase 2-mediated suppression of macrophage interleukin-12 production after thermal injury, Am. J. Physiol. Cell Physiol. 282 (2002) C263–C270.
[9] L. Yang, B. Hsu, The roles of macrophage (M phi) and PGE-2 in postburn immunosuppression, Burns 18 (1992) 132–136.
[10] J.L. Ninnemann, Prostaglandins, Leukotrienes, and the Immune Response, Cambridge University Press, Cambridge, 1988.
[11] V. Shreedhar, T. Giese, V.W. Sung, S.E. Ullrich, A cytokine cascade including prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression, J. Immunol. 160 (1998) 3783–3789.
[12] E. Louis, D. Franchimont, M. Deprez, A. Lamproye, N. Schaaf, P. Mahieu, J. Belaiche, Decrease in systemic tolerance to fed ovalbumin in indomethacin- treated mice, Int. Arch. Allergy Immunol. 109 (1996) 21–26.
[13] W.V. Scheuer, M.V. Hobbs, W.O. Weigle, Interference with tolerance induction in vivo by inhibitors of prostaglandin synthesis, Cell. Immunol. 104 (1987) 409–418.
[14] R.D. Newberry, W.F. Stenson, R.G. Lorenz, Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen, Nat. Med. 5 (1999) 900–906.
[15] P. Kalinski, C.M. Hilkens, A. Snijders, F.G. Snijdewint, M.L. Kapsenberg, Dendritic cells, obtained from peripheral blood precursors in the presence of PGE2, promote Th2 responses, Adv. Exp. Med. Biol. 417 (1997) 363–367.
[16] G. Strassmann, V. Patil Koota, F. Finkelman, M. Fong, T. Kambayashi, Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2, J. Exp. Med. 180 (1994) 2365–2370.
[17] T.C.T.M.V. Kraan, L.C.M. Boeije, R.J.T. Smeenk, J. Wijdenes, L.A. Aarden, Prostaglandin-E2 is a potent inhibitor of human interleukin-12 production, J. Exp. Med. 181 (1995) 775–779.
[18] C.L. Karp, M. Wysocka, X. Ma, M. Marovich, R.E. Factor, T. Nutman, M. Armant, L. Wahl, P. Cuomo, G. Trinchieri, Potent suppression of IL-12 production from monocytes and dendritic cells during endotoxin tolerance, Eur. J. Immunol. 28 (1998) 3128–3136.
[19] V. Donckier, V. Flamand, F. Desalle, M.L. Vanderhaeghen, M. deVeerman, K. Thielemans, D. Abramowicz, M. Goldman, IL-12 prevents neonatal induction of transplantation tolerance in mice, Eur. J. Immunol. 28 (1998) 1426–1430.
[20] A.M.E. Claessen, B.M.E. Vonblomberg, J. Degroot, D.A.E. Wolvers, G. Kraal, R.J. Scheper, Reversal of mucosal tolerance by subcutaneous administration of interleukin-12 at the site of attempted sensitization, Immunology 88 (1996) 363–367.
[21] B.M. Segal, D.M. Klinman, E.M. Shevach, Microbial products induce autoimmune disease by an IL-12-dependent pathway, J. Immunol. 158 (1997) 5087–5090.
[22] M. Maurer, W. Seidel-Guyenot, M. Metz, J. Knop, K. Steinbrink, Critical role of IL-10 in the induction of low zone tolerance to contact allergens, J. Clin. Invest. (2003).
[23] J.V. Spencer, K.M. Lockridge, P.A. Barry, G. Lin, M. Tsang, M.E. Penfold, T.J. Schall, Potent immunosuppressive activities of cytomegalovirus- encoded interleukin-10, J. Virol. 76 (2002) 1285–1292.
[24] A. Sundstedt, E.J. O'Neill, K.S. Nicolson, D.C. Wraith, Role for IL-10 in suppression mediated by peptide-induced regulatory T cells in vivo, J. Immunol. 170 (2003) 1240–1248.
[25] H. Groux, A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J.E. deVries, M.G. Roncarolo, A CD4(+) T-cell subset inhibits antigen-specific T-cell responses and prevents colitis, Nature 389 (1997) 737–742.
[26] K. Steinbrink, M. Wolfl, H. Jonuleit, J. Knop, A.H. Enk, Induction of tolerance by IL-10-treated dendritic cells, J. Immunol. 159 (1997) 4772–4780.
[27] A. Mantovani, S. Sozzani, M. Locati, P. Allavena, A. Sica, Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes, Trends Immunol. 23 (2002) 549–555.
[28] A. Mantovani, B. Bottazzi, F. Colotta, S. Sozzani, L. Ruco, The origin and function of tumor-associated macrophages, Immunol. Today 13 (1992) 265–270.
[29] F. Colotta, A. Borre, J.M. Wang, M. Tattanelli, F. Maddalena, N. Polentarutti, G. Peri, A. Mantovani, Expression of a monocyte chemotactic cytokine by human mononuclear phagocytes, J. Immunol. 148 (1992) 760–765.

[30] L. Zeng, S. An, E.J. Goetzl, Independent down-regulation of ep(2) and ep(3) subtypes of the prostaglandin E(2) receptors on U937 human monocytic cells, Immunology 86 (1995) 620–628.
[31] P. Kalinski, J. Schuitemaker, M.L. Kapsenberg, Reciprocal regulation of IL-12 p40 versus IL-12 p70 production in dendritic cells. Regulation of IL-12 subunits by PGE(2) and IL-4, J. Leukoc. Biol. (1998) H2.
[32] K. Steinbrink, L. Paragnik, H. Jonuleit, T. Tuting, J. Knop, Induction of dendritic cell maturation and modulation of dendritic cell- induced immune responses by prostaglandins [In Process Citation], Arch. Dermatol. Res. 292 (2000) 437–445.
[33] P. Kalinski, P.L. Vieira, J.H. Schuitemaker, E.C. de Jong, M.L. Kapsenberg, Prostaglandin E(2) is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer, Blood 97 (2001) 3466–3469.
[34] J.E. Buhlmann, A.H. Sharpe, The role of costimulation in T cell differentiation, in: V.K. Kuchroo, N. Sarvetnick, D.A. Hafler, L.B. Nicholson (Eds.), Cytokines and the Immune Response, Humana Press, Totowa, NJ, USA, 2002, pp. 79–118.
[35] J. Rugtveit, A. Bakka, P. Brandtzaeg, Differential distribution of B7.1 (CD80) and B7.2 (CD86) costimulatory molecules on mucosal macrophage subsets in human inflammatory bowel disease (IBD), Clin. Exp. Immunol. 110 (1997) 104–113.
[36] J.A. Hamilton, GM-CSF in inflammation and autoimmunity, Trends Immunol. 23 (2002) 403–408.
[37] P. Kalinski, J.H.N. Schuitemaker, C.M.U. Hilkens, M.L. Kapsenberg, Prostaglandin E-2 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. 161 (1998) 2804–2809.
[38] J.L. Humes, R.W. Bonney, L. Pelus, M.E. Dahlgren, S.S. Sadowski, F.A. Kuehl, Macrophages synthesize and release prostaglandins in response to inflammatory stimuli, Nature 269 (1977) 149–151.
[39] C. Zelle-Rieser, R. Ramoner, E. Artner-Dworzak, A. Casari, G. Bartsch, M. Thurnher, Human monocyte-derived dendritic cells are deficient in prostaglandin E2 production, FEBS Lett. 511 (2002) 123–126.
[40] M. Rampart, J. Van Damme, L. Zonnekyn, A.G. Herman, Granulocyte chemotactic protein/interleukin-8 induces plasma leakage and neutrophil accumulation in rabbit skin, Am. J. Pathol. 135 (1989) 21–25.
[41] K.D. Rainsford, Inhibitors of eicosanoids, in: P. Curtis-Prior (Ed.), The Eicosanoids, Wiley, Chichester, 2004, pp. 189–210.
[42] G. Tiegs, J. Barsig, B. Matiba, S. Uhlig, A. Wendel, Potentiation by granulocyte macrophage colony-stimulating factor of lipopolysaccharide toxicity in mice, J. Clin. Invest. 93 (1994) 2616–2622.
[43] M. Kreutz, B. Hennemann, U. Ackermann, E. Grage-Griebenow, S.W. Krause, R. Andreesen, Granulocyte-macrophage colony-stimulating factor modulates lipopolysaccharide (LPS)-binding and LPS-response of human macrophages: inverse regulation of tumour necrosis factor-alpha and interleukin-10, Immunology 98 (1999) 491–496.
[44] J. Xu, R. Lucas, M. Schuchmann, S. Kuhnle, T. Meergans, A.P. Barreiros, A.W. Lohse, G. Otto, A. Wendel, GM-CSF restores innate, but not adaptive, immune responses in glucocorticoid-immunosuppressed human blood in vitro, J. Immunol. 171 (2003) 938–947.