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′