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The prostaglandin E_1 analogue, Misoprostol, regulates inflammatory cytokines and immune functions *in vitro* like the natural prostaglandins E_1, E_2 and E_3

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SUMMARY

We examined whether some immune functions related to the action and production of cytokines could be regulated by the natural prostaglandins E (PGE) and the PGE_1 (ester) analogue, Misoprostol. $PGE_{1,2,3}$ and Misoprostol inhibited: (1) the mitogenic activity of interleukin-1 (IL-1) for mouse thymocytes; (2) spreading of mouse macrophages on glass; (3) tumour necrosis factor (TNF) (α and β) production by human peripheral blood mononuclear cells and rat macrophages; (4) IL-1 production by rat and mouse peritoneal macrophages; and (5) interferon-gamma (IFN-γ) production by human peripheral blood mononuclear cells. These PGE had little effect on IL-1 production by human monocytes. By contrast, they all enhanced IL-6 production by rat and mouse macrophages and human monocytes. These effects were noted at concentrations below 500 nM (even as low as 10 nM). The relative potency of the prostanoids tested for both inhibitory and stimulatory effects was $PGE_1 = PGE_2 =$ or $> PGE_3 >$ Misoprostol $> PGA_2 \gg PGF_1\text{-}\alpha = PGF_2\text{-}\alpha = PGD_2$ (no effect). There is strong evidence that $PGE_{1,2,3}$ and Misoprostol bind to the same receptor(s) and trigger the second messenger, cAMP, since dibutyryl cAMP (a lipophilic analogue of cAMP) had the same effects as the PGE. These PGE also induced elevated intracellular cAMP levels in and competed with $[^3H]PGE_2$ for binding to human and rat cells with the same relative potencies as described above.

INTRODUCTION

The products of arachidonate (20:4w6) oxygenation, in particular the monocyclic prostanoids, are known to play a central role in inflammation. Furthermore a variety of multifunctional cytokines, such as interleukin-1(IL-1), tumour necrosis factor-alpha (TNF-α) and IL-6 are considered to be important in the development and maintenance of inflammation.[1,2] It is, therefore, not surprising that anti-inflammatory therapies are often directed towards suppressing the production of prostanoids (PG) and/or inflammatory cytokines.

Therapy directed towards modifying PG production largely depends on non-steroidal anti-inflammatory drugs (NSAID). More recently, dietary supplements have been studied to attain the same goal, with the possible advantage that they might provide the same benefits as NSAID but have less side-effects.

Abbreviations: bt_2cAMP, dibutyryl cAMP; bt_2cGMP, dibutyryl cGMP; ED_2, mean twofold stimulatory dose; HBS, Hanks' buffered saline; ID_{50}, mean 50% inhibitory dose; IFN, interferon; IL, interleukin; MNP, mononuclear phagocytes; NSAID, non-steroidal anti-inflammatory drugs; PBM, peripheral blood mononuclear leucocytes; PEM, peritoneal macrophages; PHA, phytohaemagglutinin; PG, prostaglandin; TNF, tumour necrosis factor.

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Fish oil supplements (rich in eicosapentaenoic acid and docosahexaenoic acid) have been under trial for the treatment of rheumatoid arthritis.[3,4] Diets enriched with gamma-linolenic acid have also been suggested to be beneficial.[5] These diets raise the levels of precursors of PGE_1 and PGE_3 namely dihomo-gamma-linolenic acid (20:3w3) and eicosapentaenoic acid (20:5w3) respectively which compete with arachidonate for the enzyme cyclooxygenase. The net effect would be to increase the production of PGE_1 and PGE_3 possibly at the expense of PGE_2. Such changes in the relative levels of PG may have profound effects on the course of inflammation[5] depending upon the outcome of the widely known proinflammatory and anti-inflammatory effects of PG.[6]

The biologically related cytokines IL-1, TNF and IL-6 are also targets for drug therapy. These cytokines have many activities in common, can antagonize the action of one another, and further induce the production of each other and themselves.[1] Cells of the monocyte/macrophage lineage not only produce IL-1, TNF-α and IL-6 but also PG (normally PGE_2) following an inflammatory stimulus. It is important that the inter-relationships between these cytokines and PG be clarified to understand how to maximize the beneficial effects from both novel and traditional drug therapies.

We have compared the effects of PGE_1, PGE_2 and PGE_3 on (1) the production by mononuclear phagocytes, and (2) the

action, of the three inflammatory cytokines IL-1, TNF and IL-6. We have also compared their effects with the immunoregulatory/anti-inflammatory action of the more stable methyl ester of a PGE_1 analogue, Misoprostol. The parent acid of this analogue has a much longer half-life *in vivo* than natural prostaglandin E (acids). Misoprostol is currently employed as an anti-ulcer drug to treat gastric injury caused by NSAID.

MATERIALS AND METHODS

Chemicals

PGE_1, PGE_2, PGA_2, PGF_1-α, PGF_2-α, PGD_2, dibutyryl cAMP (bt_2cAMP), dibutyryl cGMP (bt_2cGMP) and Piroxicam were purchased from the Sigma Chemical Co. (St Louis, MO); and PGE_3 from Cayman Chemical Co. (Ann Arbor, MI). Misoprostol was a gift from the G. D. Searle Co. (Skokie, IL).

Cytokines

Human recombinant TNF and IL-6 were purchased from Genzyme (Cambridge, MA) and Boehringer Mannheim GmbH (Mannheim, Germany). Human recombinant IL-1 was a gift from Otsuka Pharmaceutical Co. Ltd (Tokushima, Japan).

Isolation of cells

Peritoneal macrophages (PEM) were isolated from Dark Agouti (DA) rats and C3H/HeJ mice as previously described,[7] resuspended in RPMI-1640 medium (Flow Laboratories, Irvine, Ayrshire, U.K.) supplemented with 10% foetal calf serum (FCS), penicillin 50 IU/ml and streptomycin 50 U/ml and adjusted to a concentration of 1×10^6/ml. One hundred microlitres of the cell suspension (1×10^5 cells) was placed in wells of a flat-bottomed 96-well plastic culture tray. After incubation at 37° in 5% CO_2 for 1 hr, cells not adhering to the bottom of the plastic wells were removed by washing three times with Hanks' buffered saline (HBS).

Human peripheral blood mononuclear cells (PBM) from healthy volunteers were isolated using Ficoll–Hypaque as previously described.[8]

Rodent PEM and human PBM were incubated in the presence of the test compounds in a final volume of 250 μl. In the assays for cell spreading and cytokine production, 20 μM Piroxicam was included to inhibit endogenous PGE_2 production by the MNP[9] so only the effects of the added PG would be assessed. 5 μg/ml lipopolysaccharide (LPS) (*Escherichia coli* 0111:B4, Sigma) was used to stimulate cytokine production in the experiments measuring IL-1 and TNF production. After 24 hr the supernatants were collected and stored at −70° until assayed for cytokines.

Biological assays for cytokines

The lymphocyte activating factor assay was carried out as described previously.[8]

A relatively specific (cytotoxic) IL-1 assay was conducted using the IL-1-sensitive A375 cell line as described previously.[10]

TNF was assayed similarly using the TNF-sensitive cell line L929 as described previously.[11] In our hands this cell line was sensitive to both recombinant human TNF-α and β.

To assay IL-6, 7TD1 hybridoma cells were used as previously described.[12]

In these cytokine bioassays 1 U/ml of activity was determined to be that present in a dilution of test sample which gave 50% of maximal activity. The appropriate recombinant cytokine was included in every assay to monitor variations between assays carried out at different times.

Other biological assays

The assessment of macrophage spreading on glass cover slips was carried out as previously described.[13]

Immunoassay for IFN-γ

Human PBM cells were isolated as described above. 2×10^5 of these cells were incubated in a volume of 250 μl of RPMI with various concentrations of the PG and 5 μg/ml PHA. After 24 hr the supernatant was sampled and assayed for human IFN-γ with a commercial enzyme immunoassay kit (Commonwealth Serum Laboratories, Melbourne, Australia).

Inhibition of [^{3}H]PGE_2 binding assay

Human PBM cells and rat PEM were isolated as described above. 10×10^6 cells were incubated in wells with 5 nM [^{3}H]PGE_2 (Amersham, code TRK 431) and various PG at a concentration of 50 nM in 500 μl RPMI media. After 30 min incubation at 37° in 5% CO_2 the cells were washed three times in cold HBS. The cells were lysed in Opti Phase 'Hi Safe 3' scintillation fluid (LKB, Loughborough, U.K.) to determine ^{3}H in a scintillation counter. Background binding was determined to be the ^{3}H bound in the presence of 2000-fold excess of non-radioactive PGE_2 (10 μM). Maximum binding (100%) was determined to be the ^{3}H bound in the absence of competing PG.

Induction of intracellular cAMP

Induction and measurement of intracellular cAMP was carried out as described previously[14] using a cAMP assay kit (Amersham International, Amersham, Bucks, U.K., code TRK 432).

Statistics

The level of significance was determined to be $P < 0.05$ as calculated using an unpaired, two-tail Student's *t*-test.

RESULTS

The effect of PGE and cyclic nucleotides on macrophage morphology

These prostanoids affected the morphology of mouse (C3H/HeJ) macrophages (Fig. 1). They all inhibited the speading of glass-adherent macrophages with ID_{50} (±SE) of 41·3±18·3 nM for PGE_1, 24·1±18·6 nM for PGE_2, 353±96 nM for PGE_3 and 630±178 nM for Misoprostol. Bt_2cAMP also inhibited spreading (ID_{50} = 63·3±23·4 μM) whereas bt_2cGMP enhanced the already high levels of background spreading in the presence of 20 μM Piroxicam.

The effect of PGE and cyclic nucleotides on IL-1 action

The effect of prostanoids on the lymphoproliferative action of human recombinant IL-1 was assessed using the LAF assay. PGE_1, PGE2, PGE_3 and Misoprostol were all effective inhibitors of the IL-1-induced proliferation of mouse thymocytes (Fig. 2). PGE_1 and PGE_2 were the most effective, inhibiting 50% of the activity (ID_{50}) of 10 U/ml of IL-1β at 4·6±2·7 nM and 8·6±4·2 nM respectively; PGE_3 and Misoprostol were less

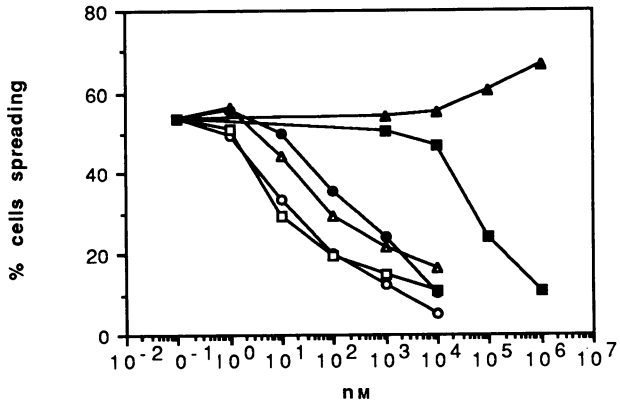

Figure 1. The effect of PG and cyclic nucleotides on spreading of murine peritoneal macrophages cultured in the presence of 20 μM Piroxicam and test compounds for 24 hr. The cells were fixed, stained and the percentage of cells spreading determined. Each point represents the mean of at least three experiments. PGE_1 (○); PGE_2 (□); PGE_3 (△); MPL (●); bt_2cAMP (■); bt_2cGMP (▲).

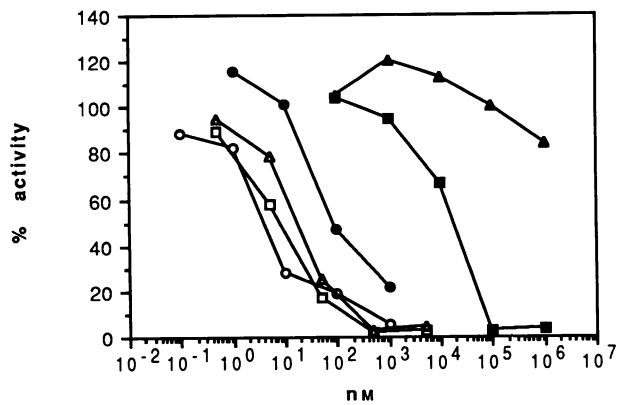

Figure 2. The effect of PG and cyclic nucleotides on the mitogenic effect of IL-1. The lymphoproliferative response of mouse thymocytes was measured by the uptake of [^{3}H]thymidine in the presence of 10 U/ml of recombinant human IL-1, suboptimal levels of PHA (1 μg/ml) and PG or cyclic nucleotides. Each point represents the mean of four experiments. PGE_1 (○); PGE_2 (□): PGE, (△); MPL (●); bt_2cAMP (■); bt_2cGMP (▲).

effective with ID_{50} of 17·0 ± 1·5 nM and 87 ± 13·0 nM respectively. Bt_2cAMP also inhibited IL-1 activity (ID_{50} = 1·97 ± 1·86 μM) whereas bt_2cGMP had no effect at the concentrations tested (10^2 to 10^6 nM).

The effect of Misoprostol on cytokine production

Figure 3 shows the effect of Misoprostol on the TNF, IL-1, IL-6 and IFN-γ activity produced by (1) human PBM, (2) murine and (3) rat PEM. Misoprostol greatly inhibited the production of TNF (Fig. 3a) by human PBM and rat PEM. The mouse strain used (C3H/HeJ) produced no detectable TNF activity. Figure 3b shows the effect of Misoprostol on IL-1 production. We consistently found a slight increase in IL-1 activity produced by

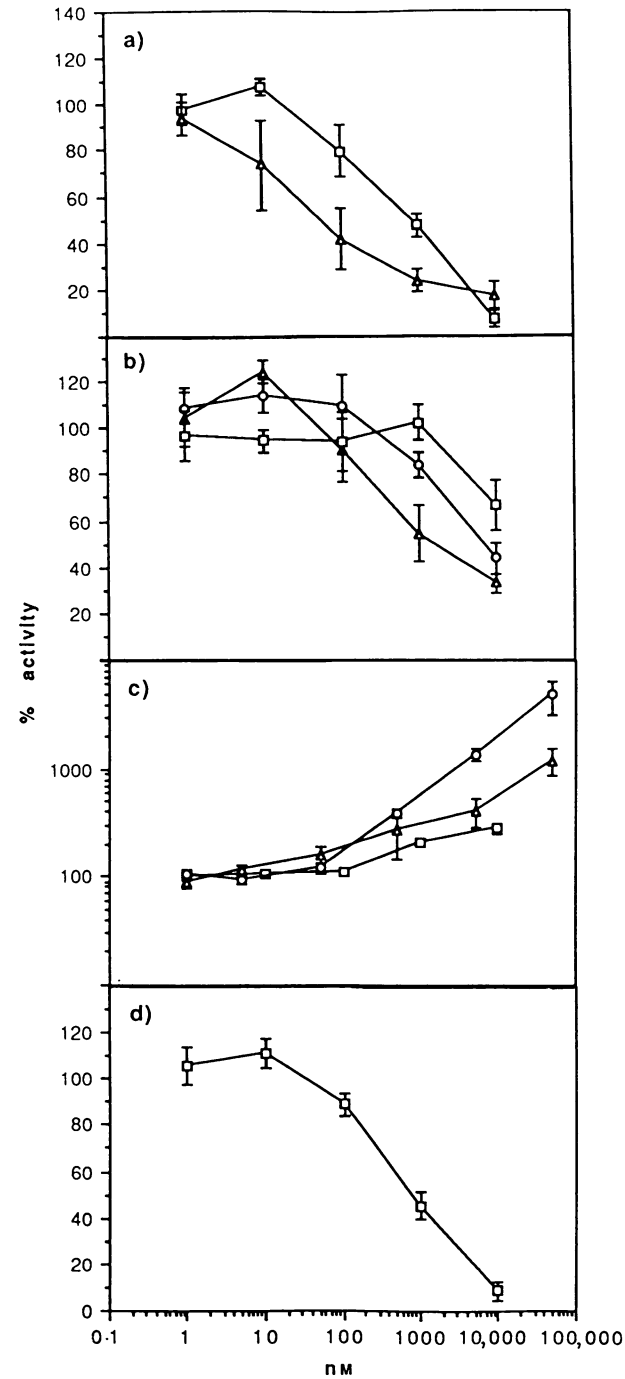

Figure 3. The effect of Misoprostol on (a) TNF, (b) IL-1, (c) IL-6 and (d) IFN-γ production by human PBM and rodent PEM. Production of TNF and IL-1 was stimulated with 5 μg/ml LPS, production of IFN-γ was stimulated with 5 μg/ml PHA and no stimulus was used for IL-6. Cells were incubated with Misoprostol at various concentrations for 24 hr, then supernatants were sampled and their cytokine content measured as described in Materials and Methods. Each point represents the mean ± SE of data obtained from experiments using cells from at least four human donors (□) and at least three experiments for mice (○) and rats (△). One hundred per cent activity represents the activity of cytokine produced by the cells in the absence of Misoprostol.

Table 1. Inhibitory effect of prostaglandin E on the production of (a) TNF, (b) IL-1 and (c) IFN-γ activity* by three types of mononuclear phagocytes

Treatment	ID_{50} (nM)†		
	Human PBM	Mouse PEM	Rat PEM
(a) *TNF*			
PGE_1	43·1 ± 16·1‡	ND§	17·3 ± 8·2
PGE_2	44·3 ± 20·2	ND	34·5 ± 11·0
PGE_3	126·6 ± 60·3	ND	274·6 ± 95·7
MPL	750·7 ± 256·6	ND	232·5 ± 69·1
bt_2cAMP	18,300 ± 6300	ND	71,300 ± 9670
(b) *IL-1*			
PGE_1	> 1000	811 ± 261	16·6 ± 3·0
PGE_2	> 1000	953 ± 348	12·7 ± 3·1
PGE_3	> 1000	1123 ± 313	29·8 ± 8·0
MPL	> 10,000	7100 ± 1740	812 ± 236
bt_2cAMP	692,000 ± 186,000	456,000 ± 49,500	41,200 ± 2400
(c) *IFN-γ*			
PGE_1	29·8 ± 11·3	—¶	—
PGE_2	33·1 ± 10·2	—	—
PGE_3	39·3 ± 13·3	—	—
MPL	591 ± 168	—	—
bt_2cAMP	29,700 ± 5070	—	—

* As measured in (a) L929 cytotoxicity assay, (b) A375 cytotoxicity assay and (c) IFN-γ immunoassay.

† Concentration (nM) which inhibited by 50% the activity of cytokine produced after stimulation with 5 μg/ml LPS (for IL-1 and TNF) or 5 μg/ml PHA (for IFN-γ). Values were derived from dose-response curves based on data derived from the means of duplicate experiments, using at least five donors for human PEM and three separate experiments using animal PEM. Endogenous PG production was suppressed with 20 μM Piroxicam (see Materials and Methods).

‡ Mean ± SE.

§ ND, not detectable following LPS stimulation of C3H/HeJ mouse peritoneal macrophages.

¶ Not tested.

Table 2. Stimulatory effect of prostaglandin E on the production of IL-6 activity* by three types of mononuclear phagocytes

Treatment	ED_2 (nM)†		
	Human PBM	Mouse PEM	Rat PEM
PGE_1	180 ± 45·2‡	23·0 ± 8·3	24·2 ± 9·5
PGE_2	417 ± 160	7·1 ± 3·0	20·2 ± 0·3
PGE_3	463 ± 121	180 ± 12·6	170 ± 28·9
MPL	966 ± 225	140 ± 38·3	346 ± 76·7
bt_2cAMP	437,000 ± 186,000	42,600 ± 9650	60,000 ± 2900

* As measured in a mitogenic assay using 7TD1 cell proliferation.

† Concentration (nM) which stimulated the activity of IL-6 produced twofold (ED_2) above control levels. Each value derived from the dose-response curves based on means of duplicate experiments, using at least five donors for human PBM and three experiments for animal PEM. Experiments were carried out in the presence of 20 μM Piroxicam (see Materials and Methods).

‡ Mean ± SE.

Table 3. Inhibition of $[^3H]PGE_2$ binding and stimulation of intracellular cyclic AMP production by prostaglandin E and Misoprostol

PG	% inhibition*		cAMP (nM)†	
	Human PBM	Rat PE	Human PBM	Rat PE
Untreated	0	0	1·43 ± 0·35	0·01 ± 0·00
PGE_1	79·9 ± 12·3	86·4 ± 5·4	13·8 ± 5·12	0·19 ± 0·05
PGE_2	85·6 ± 9·4	88·0 ± 3·5	14·6 ± 2·08	0·12 ± 0·11
PGE_3	88·5 ± 7·6	90·4 ± 6·4	14·5 ± 3·11	0·15 ± 0·08
MPL	47·2 ± 6·9	73·6 ± 8·8	7·00 ± 0·72	0·08 ± 0·07

* Per cent inhibition of binding of $[^3H]PGE_2$ by a 10-fold excess (50 nM) of PG. Mean ± SE of three experiments.

† Picomoles cAMP produced per 2×10^5 cells. Mean ± SE of three experiments.

rat and mouse macrophages with the lowest concentrations of Misoprostol (1–10 nM). This effect was also noted when the other PGE were added to rodent PEM but not observed in experiments with human PBM. Misoprostol stimulated IL-6 activity in the three cell types (Fig. 3c). Misoprostol was most effective at stimulating rat and mouse peritoneal macrophages. Misoprostol also inhibited the levels of IFN-γ produced by human PBM (Fig. 3d).

The effect of PGE and cyclic nucleotides on cytokine production

Table 1 compares the inhibition (ID_{50}) of the PGE and bt_2cAMP upon the activity of TNF, IL-1 (detected in bioassays) and IFN-γ (detected in an immunoassay) in the three cell types. The biological activity of TNF produced by stimulating these cells with LPS was inhibited by these four prostanoids and bt_2cAMP. IL-1 activity produced by human PBM was not significantly affected by exogenous PG. These results were confirmed by immunoassays (Genzyme kit) for IL-1 (data not shown). However production of IL-1 by mouse, and to an even greater extent rat, macrophages was reduced by these PGE. Dibutyryl cAMP at concentrations greater than 670 μM reduced IL-1 activity produced by human PBM by more than 50%, IFN-γ production could only be measured in experiments with human cells since the immunoassay used was specific for human IFN-γ. Like TNF production IFN-γ production was inhibited by all four PGE and bt_2cAMP. Summarizing the results given in Table 1, (1) PGE_1 and PGE_2 were just as effective at inhibiting TNF, IL-1 and IFN-γ production by the cell types tested; (2) PGE_3 was slightly less effective; and (3) Misoprostol was always markedly (often 10–40) times) less effective than PGE_1 and PGE_2.

In contrast to other cytokines, the (mitogenic) activity of IL-6 present in the supernatants from all three cell types was *enhanced* after treated the cells with the prostanoids or bt_2cAMP (Table 2). This stimulation of IL-6 production was observed without LPS stimulation. The relative (stimulant) potencies of the PGE was very similar to that noted for their inhibitory effects on the production of other cytokines (IL-1, TNF and IFN-γ).

Competition of PGE with [^{3}H]PGE$_2$ for cell binding

Table 3 shows that the binding of [^{3}H]PGE$_2$ (5 nM) to human PBM and rat PEM can be markedly inhibited by a 10-fold excess of cold PG (50nM). PGE$_1$, PGE$_2$ and PGE$_3$ were all able to inhibit approximately 80% of [^{3}H]PGE$_2$ binding to human PBM. Misoprostol was signifantly ($P<0{\cdot}005$) less effective, inhibiting 47% of [^{3}H]PGE$_2$ binding. Inhibition of [^{3}H]PGE$_2$ binding to rat cells was affected more by all the PGE tested. As noted with human cells Misoprostol was significantly ($P<0{\cdot}05$) less effective than the other PG.

Stimulation of intracellular cAMP by PGE

The effect of the PGE on intracellular levels of cAMP in human PBM and rat PEM is also shown in Table 3. All PGE markedly increased the levels of cAMP. In human PBM, PGE$_1$, PGE$_2$ and PGE$_3$ increased the levels of cAMP approximately 10-fold whilst Misoprostol increased cAMP levels approximatley five-fold. All the PGE had a similar effect on rat peritoneal cells; but the levels of cAMP detected were approximately 100-fold less than those detected in a similar number of human cells.

Preliminary experiments with four other PG (PGA$_2$, PGF$_1$-α, PGF$_2$-α and PGD$_2$) showed that they were poor mimics of PGE in regulating the above assays. Only PGA$_2$ had any significant effect at concentrations below 1000 nM (data not shown).

DISCUSSION

The ability of PGE$_1$, PGE$_3$ and Misoprostol to inhibit spreading of rodent PEM *in vitro* was similar to that described for PGE$_2$.[13] The relative intracellular levels of cAMP and cGMP seem to be important in determining macrophage morphology. Bt$_2$cGMP increased the already high basal level of spreading noted, whereas bt$_2$cAMP had the opposite effect, indicating the likely importance of these two second messengers in the regulation of macrophage morphology.

PGE$_2$ may inhibit lymphocyte proliferation induced by a variety of stimulants *in vitro* and *in vivo*.[9] This is largely due to its inhibition of IL-2 production by lymphocytes.[9] Both PGE$_1$ and PGE$_3$, which may be derived from dietary fatty acids, and the PGE$_1$ analogue Miroprostol, suppressed lymphocyte proliferation *in vitro*, indicating their potential use as suppressants of lymphoid functions *in vivo*.

Cytokine production of TNF, IL-1 and IL-6 were assessed by biological assays in this study. The biological activity expressed represents the sum of the actions of (1) the cytokine produced and (2) any inhibitors also present in the test sample. We therefore selected assays which are considered relatively specific and not likely to be affected by PG present in the test samples. However, we cannot rule out the effects of other, as yet, unidentified factors that might also be regulated by any PG present in the test supernatants. It is, however, the total biological activity produced (cytokine plus inhibitor) which will determine the final effects of PG on the cytokine-mediated immune responses.

The inhibitory effect of PGE$_2$ upon TNF-β production has been well documented.[15,16] This inhibition occurs predominantly, if not totally, at the level of mRNA production.[16] We found that PGE$_1$, PGE$_3$ and Misoprostol also inhibit the activity of TNF produced by human mononuclear cells and rat peritoneal macrophages.

There has been some confusion in the literature concerning the ability of PGE$_2$ to regulate IL-1 production. It was initially reported that IL-1 production was inhibited by PGE$_2$.[17] Our experiments using human PBM indicate that PGE$_1$, PGE$_2$, PGE$_3$ and Misoprostol did not significantly affect IL-1 production detected using the A375 cell assay. However, when we used other, less specific, assays to detect IL-1 (e.g. LAF assay or the EL4-nob1/ CTLL cell assay),[18] the apparent IL-1 activity was markedly reduced following treatment with PGE. This was probably due to the effects of the PG on the bioassays used to detect IL-1 activity (Fig. 2).[19] Dialysis of the supernatants only partially altered this inhibition (D. R. Haynes, unpublished data) possibly indicating that PGE were only incompletely removed. Recent reports suggest that PGE$_2$ does not affect the levels of IL-1 mRNA produced by human monocytes[20] or mouse macrophages.[16]

In contrast to the results obtained with human cells, activity of IL-1 produced by rodent macrophages was reduced by PGE as measured in our bioassay. Whilst high levels of TNF might also be detected in this IL-1 bioassay, it is unlikely that we measured only TNF suppression. Firstly we noted this effect using peritoneal macrophages from C3H/HeJ mice which did not produce detectable TNF (Table 1).[21] Secondly, the levels of TNF found in the rat macrophage supernatants (<20 U/ml) did not affect the action of IL-1 on A375 cells. At very low concentrations of PGE we consistently observed a slight increase in IL-1 activity indicating that PGE may possible both stimulate[22] *and* suppress depending on concentration. The effects of PGE on IL-1 production may therefore vary depending upon the species and concentrations used.

Unlike the other cytokines, IFN-γ production was measured in an immunoassay. The inhibitory effect of PGE$_2$ on IFN-γ production has been described previously and its effects on immune responses have been discussed.[23] IFN-γ has been extensively described as a stimulator of many monocyte/ macrophage functions, including inflammatory cytokine production. Therefore, suppression of IFN-γ may markedly affect progression of inflammatory diseases.

The prostanoids all stimulated IL-6 production, in marked contrast to their effects on the other cytokines investigated. This was noted at nanomolar concentrations with both rodent PE and human PBM. It is consistent with reports that PGE$_1$ and other stimulators of intracellular cAMP enhance IL-6 production in human foreskin fibroblasts.[24]

Since PGE$_1$, PGE$_2$, PGE$_3$ and Misoprostol all had similar effects on the range of biological activities investigated, they probably have the same mechanism of action and bind to the same cell surface receptor(s). The relative abilities of the PGE to compete with radiolabelled PGE$_2$ for cell binding was similar to their biological activities. This may indicate that observed differences in potency reflect their different affinities for such a receptor, i.e. PGE$_1$ = PGE$_2$ = or $>$ PGE$_3$ $>$ Misoprostol. Other PG tested (PGA$_2$, PGF$_1$-α, PGF$_2$-α and PGD$_2$) had very little effect in these assays, probably indicating they bind to different receptor types as has been reported.[25]

Cells generally respond to prostanoids by increasing the intracellular levels of cAMP, a 'second messenger' involved in the transduction of signals from membrane receptors to other sites within the cell.[26] We found that bt$_2$cAMP had the same effects as these prostanoids on macrophages/monocytes. Also, the PGE were able to increase the intracellular levels of cAMP

(in human and rat cells) with similar relative potencies to their cell binding and their effects on cytokines. This suggests that the effects of all four of the prostanoids E are mediated by their ability to increase intracellular cAMP.

Generally, we found that human PBM were less sensitive to the PGE than rodent peritoneal cells. However, rather than reflecting a species difference, this may indicate that mature peritoneal macrophages are more sensitive than immature peripheral blood monocytes and lymphocytes. This is supported by the observation that rat PE cells produce much less intracellular cAMP, either normally or following PGE treatment, than human PBM. More studies are needed to determine if this is due to increased sensitivity of the rodent to intracellular cAMP or other factors such as numbers of PGE receptors.

The ability of prostaglandin E to regulate the production of inflammatory cytokines and other MNP functions described here may be examples of natural feedback mechanisms for controlling inflammation. Suppression of MNP spreading, IL-1 action, IFN-γ, TNF and, occasionally, IL-1 production, whilst stimulating the comparatively less acutely cytotoxic cytokine IL-6, may lead to an overall reduction of inflammation. IL-6 is also a potent stimulator of acute phase protein production by the liver (reviewed in ref. 27), and a common function of these acute phase proteins may be to help restore the homeostatic balance of the inflamed tissue.[28]

These regulatory effects may be particularly relevant during the acute phase of the inflammatory process, such as active arthritis, where high levels of PGE_2 have been described.[29] These concentrations (1–100 nM) are similar to those at which we observed significant effects *in vitro*. Since MNP not only produce PGE, but also are affected by them, PGE effects need only be short ranged. Therefore, much higher effective concentrations in this microenvironment may be attained. In addition, in this study we have often assessed the PGE suppression of responses to strong stimuli (10 U/ml IL-1 and 5 μg/ml LPS), but these PGE may be even more effective at suppressing weaker stimuli.

Such a regulatory mechanism has two major implications for current and prospective therapies. Firstly, the commonly used NSAID which strongly inhibit PG production, may be effective at relieving some symptoms of inflammation (e.g. pain, vasodilation, etc.) but might not reduce the long-term progression of the disease sustained or mediated by inflammatory cytokines (e.g. IL-1, TNF, etc.). Secondly, dietary control of inflammation by fish oils, etc. may be more effective in the long term if allowed to augment the natural regulation of PGE_2 by producing additional PGE_1 or PGE_3. Conversely, if dietary treatment were to result in lower levels of total PGE (i.e. $PGE_2 + PGE_1$ or PGE_3), as has been reported,[30] the suppressive effects of PGE may be reduced. However, suppression of cytokine production by modifying the production of leukotriene B_4[31,32] or other products of lipoxygenase[33] may balance/replace the loss of PGE_2 suppression.

Misoprostol was developed to reduce the damaging effects of NSAID and other gastrotoxins on the stomach. However, recent findings suggest it may have other beneficial effects such as prolonging the survival of transplants. This may reflect the immunosuppressive[34] and anti-inflammatory[35] effects of the drug. Although the data shown in this report were generated with the ester form of Misoprostol, we found the acid form identical in activity in all the assays used (data not shown). This probably indicated that cells used in these assays contain sufficient esterase activity to generate the free acid of Misoprostol. In all our experiments, Misoprostol closely resembled the naturally occurring prostanoids, supporting the suggestion that stable synthetic PG might be useful in therapy where such immune regulation is beneficial.[36] Its use in conjunction with other therapies reducing PG production, e.g. NSAID or dietary control as described above, may be of particular value.

The results of this study indicate that PG can modify immune responses generated *in vitro* using non-specific cell stimuli. This action of PG could be further explored *in vivo* by diet enrichment with their precursor fatty acids or controlled administration of PG mimics, such as Misoprostol.

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