



# Murine myeloid progenitor responses to GM-CSF and eosinophil precursor responses to IL-5 represent distinct targets for downmodulation by prostaglandin E<sub>2</sub>

\*<sup>1</sup>Maria Ignez C. Gaspar Elsas, <sup>2</sup>Danielle Joseph, <sup>3</sup>Leticia Lintomen, <sup>3</sup>Elisabeth S. Maximiano, <sup>3</sup>Michael Bodstein, <sup>3</sup>P. Xavier Elsas & <sup>2</sup>B. Boris Vargaftig

<sup>1</sup>Depto. de Pesquisa, Instituto Fernandes Figueira, Fiocruz, Rio de Janeiro, Brazil; <sup>2</sup>Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur-INSERM U485, Paris, France and <sup>3</sup>Depto. de Imunologia, Instituto de Microbiologia, UFRJ, Rio de Janeiro, Brazil

**1** Because Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and dibutiryl cyclic AMP (dbcAMP) modulate the production and effects of haemopoietic cytokines in allergy, we examined their ability to modulate responses of myeloid progenitors to GM-CSF, and of eosinophil precursors to IL-5.

**2** The ability of PGE<sub>2</sub>, dbcAMP, rolipram, forskolin, dbcGMP and PGD<sub>2</sub>, to modulate the responses to GM-CSF and IL-5 in colony formation (progenitor) and eosinophil differentiation (precursor) assays using bone-marrow from nonsensitized or from intranasally-challenged, ovalbumin-sensitized mice of five strains was studied.

**3** PGE<sub>2</sub> (10<sup>-7</sup> M) inhibited GM-CSF-stimulated colony formation in bone-marrow from BP-2 mice. This effect was duplicated by dbcAMP (0.3–1 × 10<sup>-6</sup> M), Rolipram (10<sup>-5</sup> M) and forskolin (3 × 10<sup>-5</sup> M), but not Prostaglandin D<sub>2</sub> (10<sup>-6</sup> M). Inhibition affected similarly all myeloid colony types. Progenitors from sensitized and challenged BP-2 mice were also inhibited by PGE<sub>2</sub> and cyclic AMP. PGE<sub>2</sub> inhibited progenitors from C57BL/10, CBA/J and A/J, but not BALB/c mice. However, BALB/c progenitors were sensitive to dbcAMP and Forskolin (10<sup>-4</sup> M). In contrast, in precursor assays, PGE<sub>2</sub> (10<sup>-7</sup>–10<sup>-9</sup> M) blocked responses to IL-5 in bone-marrow from BP-2 and BALB/c mice, either naïve or sensitized and challenged, to a similar extent. PGD<sub>2</sub> (10<sup>-6</sup> M) was ineffective, as was PGE<sub>2</sub> (10<sup>-7</sup> M), if added after 48 h of culture.

**4** In conclusion, PGE<sub>2</sub> inhibits the responses of bone-marrow myeloid progenitors to GM-CSF and of eosinophil precursors to IL-5, in naïve or ovalbumin sensitized and challenged mice. These effects are duplicated by cyclic AMP-elevating agents. In the BALB/c strain, the resistance of progenitors, but not precursors, to PGE<sub>2</sub> inhibition, indicates these developmental stages are separate targets for PGE<sub>2</sub> modulation.

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**Keywords:** Prostaglandin E<sub>2</sub>; haemopoiesis; GM-CSF; IL-5; bone-marrow

**Abbreviations:** dbcAMP, dibutiryl cyclic adenosine monophosphate; GM-CSF, granulocyte-macrophage colony stimulating factor; IL-3, interleukin-3; IL-5, interleukin-5; PGD<sub>2</sub>, prostaglandin D<sub>2</sub>; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>

## Introduction

Studies on asthma in patients and in animal models have shown upregulation of responses to the haemopoietic cytokines after allergen exposure in humans (Sehmi *et al.*, 1997), mice (Gaspar Elsas *et al.*, 1997) and dogs (Inman *et al.*, 1996). Therefore, changes in the ability of haemopoietic cells to respond to cytokines is a potential target for therapeutic modulation. In a canine model, Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) blocks the upregulation by allergen-induced serum factors of the responses of haemopoietic progenitors to Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF; Inman *et al.*, 1997). Hence, it is of interest to evaluate whether PGE<sub>2</sub> similarly affects the upregulation of bone-marrow responses to eosinopoietic cytokines in the murine model, which is more convenient for genetic and immunologic studies, and also involves allergen-induced, circulating factors (Gaspar Elsas *et al.*, 1997).

Several early studies reported that different prostaglandins as well as cyclic AMP (cAMP)-elevating agents inhibited proliferation of murine (Kurland *et al.*, 1977) and human

(Taetle & Koessler, 1980) haemopoietic cells *in vitro*. Early *in vivo* studies also showed enhanced myelopoiesis associated with inhibition of prostaglandin biosynthesis in mice (Pelus, 1989), as well as induction of a myelopoiesis inhibitor in spleen and bone-marrow cultures from PGE<sub>2</sub>-injected mice (Gentile & Pelus, 1988). However, these studies are difficult to interpret, because: (a) the conditioned media used to stimulate haemopoietic cells *in vitro* contain several different, potentially interacting, growth factors; (b) the *in vivo* experiments do not address the direct effects of PGE<sub>2</sub> on haemopoietic cells, since many different cell populations, as well as PGE<sub>2</sub>-induced mediators were involved (Pelus & Gentile, 1988, Gentile & Pelus, 1988). More recently, prostaglandins were shown to down-modulate murine haemopoiesis (O'Reilly & Gamelli, 1990, Kozubik *et al.*, 1994), possibly acting through cyclic AMP-dependent mechanisms (Gamelli *et al.*, 1994). PGE<sub>2</sub> and dbcAMP directly inhibited murine GM colony formation (Gamelli *et al.*, 1994). However, prostaglandin effects on murine and human bone-marrow may still involve inhibition of colony-stimulating factor production by stromal cells (Gamelli *et al.*, 1998; Bug *et al.*, 1998). As to the eosinophil lineage, which is relevant to asthma, selective inhibition of

\*Author for correspondence; E-mail: elsas@iff.fiocruz.br

progenitors by PGE<sub>2</sub> has not been reported, but cyclic AMP-elevating agents may either up- or down-modulate human GM-CSF-dependent mature eosinophil survival (Hallsworth *et al.*, 1996).

We have reevaluated the direct effects of PGE<sub>2</sub> and cyclic AMP-elevating agents on responses to exogenously added, recombinant, eosinopoietic cytokines in murine bone-marrow. We used assays that distinguish between their effects on progenitors (colony-forming cells) and precursors (noncolony-forming, lineage-committed cells derived from the former; Bagby, 1994), because these two classes of targets are differently affected by allergen challenge: progenitors do not increase in total number, but become increasingly committed to eosinophil colony formation, while precursors yield larger numbers of terminally differentiated eosinophils (Gaspar Elsas *et al.*, 1997). Since the effects of PGE<sub>2</sub> and dbcAMP, as those of the eosinopoietic cytokines themselves, may be modified by allergen exposure, we further examined whether the ability of these agents to modulate responses to GM-CSF and to IL-5 was modified by ovalbumin-sensitization and challenge.

## Methods

### *Animals and animal procedures*

For most of the study, male and female BP-2 and BALB/c mice bred at Elevage Janvier (Le Genest Saint-Isle, France), were used at 6–8 weeks of age. BALB/c mice bred at Fiocruz, Rio de Janeiro (Brazil), yielded comparable results. A/J, CBA/J and C57BL/10 mice bred at Fiocruz were also used. BALB/c and BP-2 mice were immunized with two s.c. 0.4 ml injections of 100 µg ovalbumin mixed with 4 mg ml<sup>-1</sup> Al(OH)<sub>3</sub> in 9% NaCl, at 7 day intervals. BALB/c mice were intranasally challenged with 10 µg ovalbumin in 50 µl 0.9% NaCl, 1 week after the second injection, a procedure that increases responses to eosinopoietic cytokines but does not induce hyperreactivity (Gaspar Elsas *et al.*, 1997). In selected experiments, BP-2 mice were submitted to repeated challenges (twice a day, for 2 days), which induce intense bronchopulmonary hyperreactivity in this strain (Eum *et al.*, 1995; Haile *et al.*, 1999). All groups were sacrificed 24 h after completing the challenge procedure. Animal handling followed the standard procedures adopted at the Institut Pasteur, Paris (France).

### *Reagents*

Heat-inactivated Foetal Calf Serum (FCS), L-glutamate and culture media were from Gibco (Life Technologies SARL, Cergy Pontoise, France), Diff-Quick from DADE Diagnostika GmbH (Unterschleissheim, Germany) and Harris' Hematoxylin from Réactifs RAL (Paris, France). Recombinant rmIL-5 was from Pharmingen (San Diego, CA, U.S.A.). Dibutiryl cyclic AMP (dbcAMP), dibutiryl cyclic GMP (dbcGMP) were from Biolum Research Laboratories Inc., Plymouth, U.S.A. Forskolin was from Sigma France. Rolipram was from Sanofi Labs., Montpellier, France. Prostaglandins E<sub>2</sub> (PGE<sub>2</sub>) and D<sub>2</sub> (PGD<sub>2</sub>) were from Cayman Chemical Co., Ann Arbor, U.S.A. PGE<sub>2</sub> was prepared as a 10<sup>-3</sup> M stock solution in pure ethanol and diluted in medium before use. Control cultures with ethanol at the same final concentration found in PGE<sub>2</sub>-treated cultures were included. Isoproterenol was from Sigma and prepared as a 10<sup>-5</sup> M stock solution in medium. Ovalbumin (5 × crystallized) was from ICN Biomedicals, Inc. (Costa Mesa, CA, U.S.A.), Al(OH)<sub>3</sub> from Merck (Darmstadt, Germany).

### *Bone-marrow cell studies*

**Bone-marrow cell harvest, identification and separation** Bone-marrow cells, collected by flushing the two femurs of 5–8 mice with RPMI 1640 medium containing 10% FCS, were washed and counted. The frequency of cells stained for eosinophil peroxidase (EPO) following the protocol of Ten *et al.* (1989) was determined in cyto-centrifuge smears (Gaspar Elsas *et al.*, 1997). The cytochemical pattern of EPO+ cells in stained bone-marrow preparations was identified to that described by Horton *et al.* (1996).

**Progenitor assays** 1 ml semi-solid cultures were established in 35 mm culture dishes, from 2 × 10<sup>5</sup> cells, at least in triplicate, in a mixture of Iscove's modified Dulbecco's medium, with 20% FCS and agar to 0.3% final concentration (Kurland *et al.*, 1977; Gaspar Elsas *et al.*, 1997), with GM-CSF at 2 ng ml<sup>-1</sup>, alone or in association with any of the following: PGE<sub>2</sub>, PGD<sub>2</sub>, Rolipram, forskolin, isoproterenol, dbcAMP, or dbcGMP, at the various concentrations detailed in Results. A colony was defined as progenitor-derived ensemble larger than 50 cells (Bagby, 1994). Colonies were scored at day 7 under the inverted microscope at low magnification, and the frequency of eosinophil colonies was determined on agar layers dried (50°C), mounted on microscope slides, stained for EPO, and scored under high magnification (Gaspar Elsas *et al.*, 1997). We have previously confirmed that these conditions were adequate for counting total myeloid colonies and for accurate differential counts of myeloid colony types on dried agar layers (Gaspar Elsas *et al.*, 2000).

**Precursor assays** Liquid bone-marrow cultures (10<sup>6</sup> cells in a 1 ml volume, in a 24-well cluster, Gaspar Elsas *et al.*, 1997) were seeded in RPMI 1640 medium, with 10% FCS, 2 mM L-glutamine, and penicillin-streptomycin, at 37°C, 5% CO<sub>2</sub>, 95% air, for 7 days, at least in triplicate, with rmIL-5 (Pharmingen, 0.01–1 ng ml<sup>-1</sup>), alone or in association with PGE<sub>2</sub>, PGD<sub>2</sub>, forskolin, dbcAMP, or dbcGMP, at the various concentrations detailed in Results. The frequency of EPO+ cells was determined after 7 days of culture in cyto-centrifuge smears. We have previously confirmed that these conditions were adequate for evaluating eosinophil differentiation in both naïve and sensitized and challenged murine bone-marrow, and that the increase in per cent EPO+ cells, as directly determined on cyto-centrifuge smears, was paralleled by actual increases in the total numbers of EPO+ cells in these cultures, as indirectly determined from total and differential cell counts (Gaspar Elsas *et al.*, 2000).

**Statistical analysis** The data were analysed with the help of the Systat for Windows version 4 software, using factorial analysis of variance, and with the Tukey correction for multiple comparisons between different treatments.

## Results

### *Effect of PGE<sub>2</sub> and cAMP-elevating agents on myeloid progenitors in bone-marrow cultures from naïve BP-2 mice*

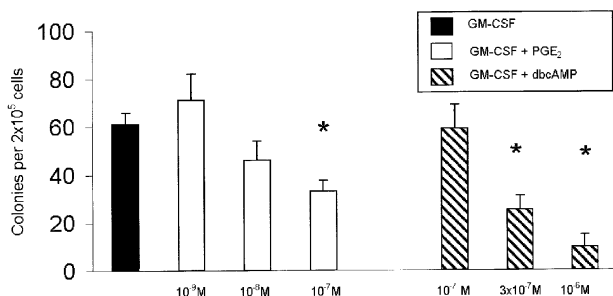
To define the effect of PGE<sub>2</sub> on colony formation by progenitors from naïve mice, semi-solid cultures were established from bone-marrow of BP-2 mice, in the presence of GM-CSF, alone or with PGE<sub>2</sub> (10<sup>-7</sup>–10<sup>-9</sup> M). As shown in Figure 1, a significant decrease in the total number of myeloid

colonies formed in the presence of GM-CSF and PGE<sub>2</sub> (10<sup>-7</sup> M) was observed, relative to the control cultures with GM-CSF alone ( $P=0.03$ ). At 10<sup>-8</sup>–10<sup>-9</sup> M, the effect of PGE<sub>2</sub> was not significant. As also shown in Figure 1, a significant decrease in myeloid colony formation was observed when cultures were established in the presence of GM-CSF and dbcAMP (3 × 10<sup>-7</sup>–10<sup>-6</sup> M), relative to the GM-CSF controls ( $P=0.007$  and  $P=0.005$ , respectively). dbcAMP at 10<sup>-7</sup> M had no effect.

As shown in Table 1, a significant decrease in the total number of myeloid colonies formed by naïve BP-2 bone-marrow cells in the presence of GM-CSF and Rolipram (10<sup>-5</sup> M) was observed, relative to the control cultures with GM-CSF alone ( $P=0.03$ ). Rolipram, at 10<sup>-6</sup>–10<sup>-7</sup> M, was ineffective. On the other hand, forskolin, at 3 × 10<sup>-5</sup> M, also inhibited significantly colony formation ( $P<0.05$ ). In contrast, Prostaglandin D<sub>2</sub>, at 10<sup>-6</sup> M, had no effect.

#### Effect of PGE<sub>2</sub> and cyclic AMP-elevating agents on myeloid progenitors in bone-marrow cultures from sensitized and challenged BP-2 mice

To define whether the ability of PGE<sub>2</sub> and dbcAMP to down-modulate GM-CSF-stimulated colony formation was modified by allergen sensitization and challenge, we established bone-marrow cultures from BP-2 mice that had been sensitized to ovalbumin and either challenged repeatedly four times over 2 days or challenged once. In BP-2 mice challenged four times, GM-CSF-stimulated colony formation was decreased from 66 ± 17 (mean ± s.e.mean) in control cultures to 20 ± 3.9 in cultures established in the presence of PGE<sub>2</sub> at 10<sup>-7</sup> M



**Figure 1** Effect of PGE<sub>2</sub> and dbcAMP on GM-CSF-induced colony formation in naïve bone-marrow. The data are mean ± s.e.mean of the number of myeloid colonies formed by bone-marrow from naïve BP-2 mice. GM-CSF was used at 2 ng ml<sup>-1</sup>. Asterisks indicate significant differences relative to the GM-CSF controls. Data are derived from 4–19 experiments.

**Table 1** Effect of cyclic AMP-elevating agents on GM-CSF-stimulated colony formation by bone-marrow of naïve BP-2 mice

	Colonies per 2 × 10 <sup>5</sup> cells	n	P
GM-CSF	46.4 ± 3.8	6	—
Rolipram, 10 <sup>-5</sup> M	17.4 ± 1.5	3	0.03
Rolipram, 10 <sup>-6</sup> M	28.7 ± 4.3	3	NS
Rolipram, 10 <sup>-7</sup> M	50.8 ± 5.3	3	NS
Forskolin, 3 × 10 <sup>-5</sup> M	26.1 ± 8.1	3	<0.05
Prostaglandin D <sub>2</sub> , 10 <sup>-6</sup> M	38.8 ± 1.6	3	NS

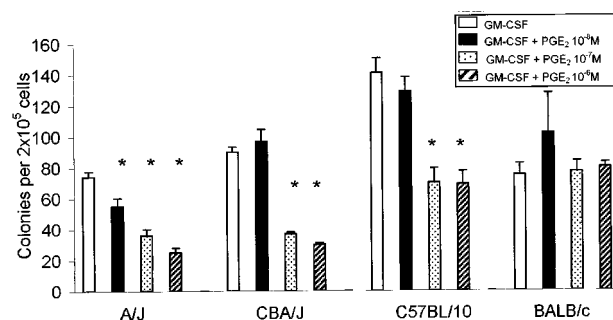
Data are mean ± s.e.mean of colony numbers formed by 2 × 10<sup>5</sup> BP-2 bone-marrow cells, derived from the indicated numbers of experiments, established in the presence of 2 ng ml<sup>-1</sup> GM-CSF, either alone or associated with the indicated concentrations of Rolipram, Forskolin and PGD<sub>2</sub>.

( $P<0.001$ ,  $n=8$ ), and to 6 ± 1.8 in cultures established with dbcAMP at 10<sup>-6</sup> M ( $P<0.001$ ,  $n=6$ ). In contrast, PGE<sub>2</sub> at 10<sup>-8</sup>–10<sup>-9</sup> M, or dibutyryl cyclic GMP (dbcGMP), at 10<sup>-6</sup> M, had no effect. Similar effects were observed in BP-2 mice challenged only once ( $P<0.001$ ).

#### Effect of PGE<sub>2</sub> and cyclic AMP-elevating agents on myeloid progenitors in naïve mice from different strains

To define whether the sensitivity to inhibition of colony formation by PGE<sub>2</sub> was dependent on the genetic background of the bone-marrow donors, we established semi-solid bone-marrow cultures from mice of the A/J, CBA/J, C57BL/10 and BALB/c strains, in the presence of GM-CSF, alone or associated with PGE<sub>2</sub> (10<sup>-7</sup>–10<sup>-9</sup> M). As shown in Figure 2, GM-CSF-stimulated colony formation, which varied from one strain to the other, was significantly inhibited in bone-marrow from the A/J, CBA/J and C57BL/10 strains ( $P<0.01$  in all cases) by addition of PGE<sub>2</sub> at one or more of the concentrations tested. The effective dose range varied from one strain to the other, and A/J bone-marrow progenitors were significantly inhibited at a concentration (10<sup>-9</sup> M) that was no longer effective for the other strains. In contrast, BALB/c progenitors were not inhibited at any of the PGE<sub>2</sub> concentrations tested, and were unaffected even by PGE<sub>2</sub> at 10<sup>-6</sup> M.

To define whether unresponsiveness to PGE<sub>2</sub> in BALB/c mice originated in a defect in cyclic AMP-dependent signalling mechanisms, we established semi-solid cultures in the presence of GM-CSF, alone or associated with PGE<sub>2</sub>, dbcAMP, forskolin or isoproterenol. Colony numbers per 2 × 10<sup>5</sup> bone-marrow cells plated were significantly decreased, from 77 ± 8.3 (mean ± s.e.mean; data are derived from 12 experiments) in the presence of GM-CSF alone, to 16 ± 5.2 ( $P=0.029$ ) when dbcAMP (10<sup>-6</sup> M) was added, while PGE<sub>2</sub> at either 10<sup>-7</sup> or 10<sup>-6</sup> M had no significant effect (92 ± 10 and 95 ± 5.4, respectively). In another series of experiments, colony formation by 2 × 10<sup>5</sup> cells was significantly decreased, from 54 ± 2.6 in control cultures (mean ± s.e.mean, data are derived from six experiments) to 25 ± 4 in cultures established in the presence of forskolin, at 10<sup>-4</sup> M ( $P<0.001$ ). In contrast, forskolin at 3 × 10<sup>-5</sup> M was ineffective. In a further series of experiments, isoproterenol at 10<sup>-6</sup>–10<sup>-11</sup> M did not significantly inhibit colony formation by BALB/c bone-marrow cells. In contrast, isoproterenol at 10<sup>-6</sup>–10<sup>-7</sup> M strongly inhibited colony formation by bone-marrow cells from C57BL/10 mice (not shown).



**Figure 2** Effect of PGE<sub>2</sub> on GM-CSF-induced colony formation in bone-marrow from different mouse strains. The data are mean ± s.e.mean of the number of myeloid colonies formed by bone-marrow from naïve mice of the indicated strains. GM-CSF was used at 2 ng ml<sup>-1</sup>. Asterisks indicate significant differences relative to the respective GM-CSF controls ( $P<0.01$  in all cases). Data are derived from 3–19 experiments.

### Lack of lineage-selectivity in progenitor inhibition by PGE<sub>2</sub> and dbcAMP

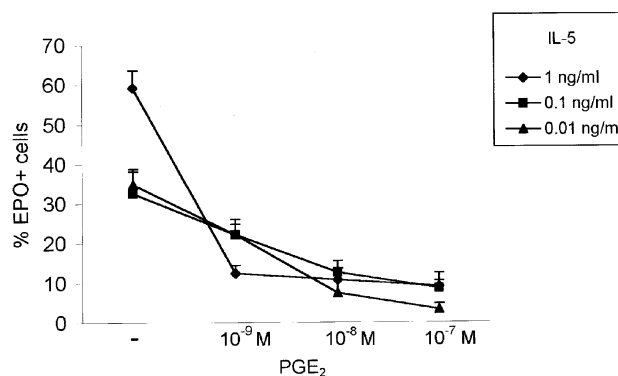
To define whether inhibition of colony formation in BP-2 bone marrow correlated with selective down-regulation of the eosinophil lineage, we performed differential colony counts on dried and stained semisolid cultures. As shown in Table 2, no significant difference in eosinophil colony frequency, either pure or mixed, was found between cultures established in the presence of GM-CSF alone and those to which PGE<sub>2</sub> had been added, at 10<sup>-7</sup>–10<sup>-8</sup> M, even though total myeloid colony number was significantly reduced in the latter (see above). No selective inhibition of GM colonies was observed. In contrast, cultures established in the presence of dbcAMP (10<sup>-6</sup> M) contained no eosinophil colonies, either pure or mixed, nor granulocyte colonies.

### Effect of PGE<sub>2</sub> and cyclic AMP-elevating agents on eosinophil precursors

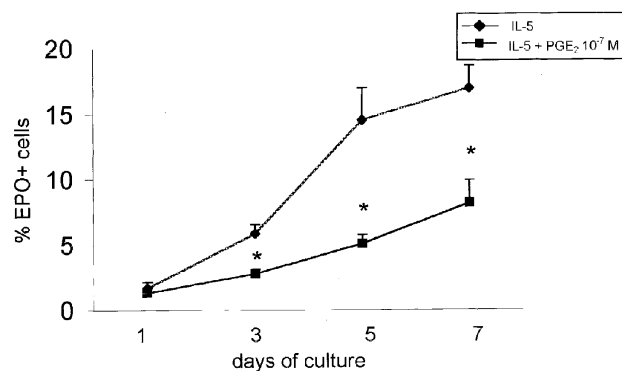
To define whether PGE<sub>2</sub> inhibited responses of eosinophil precursors to rmIL-5, liquid bone-marrow cultures were established from bone marrow of ovalbumin-sensitized and challenged (1 ×) BP-2 mice, which responds vigorously in a 7 day-eosinophil differentiation assay (Gaspar Elsas *et al.*, 1997), in the presence of various concentrations of rmIL-5 and of PGE<sub>2</sub>. As shown in Figure 3, PGE<sub>2</sub> (10<sup>-7</sup>–10<sup>-9</sup> M) inhibited IL-5 driven eosinophil differentiation for all rmIL-5 concentrations. However, the effect was most marked when 1 ng ml<sup>-1</sup> rmIL-5, which yields plateau stimulation (see Methods) was used. For further analysis, the effect of PGE<sub>2</sub> was studied at 10<sup>-7</sup> M, in the presence of 1 ng ml<sup>-1</sup> rmIL-5, unless otherwise indicated. To define whether the effect of PGE<sub>2</sub> on IL-5-driven eosinophil differentiation was immediate, bone-marrow cultures were established from BP-2 bone-marrow with rmIL-5 alone or associated with PGE<sub>2</sub> at 10<sup>-7</sup> M, and the per cent EPO+ cells was determined at various times after initiation of the culture. In parallel control cultures, bone-marrow was cultured for 7 days in the presence of rmIL-5, and PGE<sub>2</sub> was added 30 min before cells were harvested. As shown in Figure 4, EPO+ cell numbers steadily increased from day 1 to day 7 of culture, reflecting eosinophil differentiation from EPO-precursors (per cent EPO+ cells in the initial BP-2 bone-marrow inoculum was 1.5 ± 0.6, mean ± s.e.mean, *n* = 4). At 7 days of culture, plateau levels were reached in the control cultures. At day 7, EPO+ cell numbers were significantly reduced in cultures containing PGE<sub>2</sub>. Cultures containing rmIL-5 associated with effective concentrations of PGE<sub>2</sub> presented, by day 7, large numbers of viable bone-marrow derived macrophages (which do not require haemopoietic factors to survive), but had fewer morphologically intact EPO+ cells than control cultures. However, EPO+ granules could be found inside macrophages, suggesting the latter had

phagocytosed dead EPO+ cells. Cultures exposed to PGE<sub>2</sub> for only 30 min showed no decrease in EPO+ numbers relative to rmIL-5-treated controls.

To define whether unresponsiveness to PGE<sub>2</sub> in bone-marrow progenitors was associated with similar defect in eosinophil precursors, liquid cultures were established from bone-marrow of naïve or of ovalbumin-sensitized and challenged BALB/c mice, for 7 days, in the presence of



**Figure 3** Effect of PGE<sub>2</sub> on rmIL-5-driven eosinophil differentiation in bone-marrow from BP-2 mice. Data are mean ± s.e.mean of the per cent EPO+ cells in liquid cultures established from ovalbumin-sensitized and challenged BP-2 mice, with IL-5 at the indicated concentrations, without PGE<sub>2</sub> (-) or with addition of PGE<sub>2</sub> (10<sup>-7</sup>–10<sup>-9</sup> M) for 7 days. Inhibition was significant for all PGE<sub>2</sub> concentrations, relative to the respective control cultures. Data are derived from three experiments.



**Figure 4** Effect of PGE<sub>2</sub> on IL-5-driven eosinophil differentiation in bone-marrow from BP-2 mice. Data are mean ± s.e.mean of the per cent EPO+ cells in liquid cultures established from naïve BP-2 mice, in the presence of 1 ng ml<sup>-1</sup> rmIL-5, without PGE<sub>2</sub> (IL-5) or with addition of PGE<sub>2</sub> (10<sup>-7</sup> M) for the indicated number of days. Asterisks indicate significant differences relative to the respective rmIL-5 controls. Data are derived from 4–10 experiments.

**Table 2** Lack of effect of PGE<sub>2</sub> and dbcAMP on the relative frequency of different myeloid colony subtypes

	Colony type				
	M	GM	Mixed (GM+E)	G	E
GM-CSF	48 ± 3.2	27 ± 3	5 ± 1.3	6.4 ± 0.9	12.1 ± 2
+ PGE <sub>2</sub> , 10 <sup>-7</sup> M	50.4 ± 4.5	35.6 ± 4.2	1.5 ± 0.8	2.3 ± 1.3	10 ± 2.2
+ PGE <sub>2</sub> , 10 <sup>-6</sup> M	43 ± 5	38 ± 3.8	2.6 ± 0.8	6.8 ± 1.9	9.4 ± 1.8
+ dbcAMP, 10 <sup>-6</sup> M	88 ± 5.3	11.6 ± 5.3	0	0	0

Data are the per cent of each myeloid colony type as determined by differential counts on dried and stained agar layers, after culturing bone-marrow from ovalbumin-sensitized and challenged (1 ×) BP-2 mice. Data are derived from 11–12 cultures. M, macrophage; GM, granulocyte-macrophage, mixed granulocyte-macrophage with associated eosinophils; G, granulocyte; E, pure eosinophil colonies.

rmIL-5, alone or associated with PGE<sub>2</sub>. As shown in Figure 5, eosinophil differentiation occurred in the presence of rmIL-5 in bone-marrow from both naïve and immune mice. Confirming previous observations (Gaspar Elsas *et al.*, 1997), higher plateau levels were attained in cultures of sensitized and challenged bone-marrow. Addition of PGE<sub>2</sub>, at 10<sup>-6</sup>–10<sup>-8</sup> M, inhibited significantly the responses to rmIL-5 in naïve and bone-marrow ( $P < 0.003$  for all comparisons). In contrast, no inhibition was observed with PGE<sub>2</sub> at 10<sup>-9</sup> M, nor with PGD<sub>2</sub>, at 10<sup>-6</sup>–10<sup>-7</sup> M. On the other hand, bone-marrow from sensitized and challenged mice was similarly inhibited by PGE<sub>2</sub> at 10<sup>-7</sup>–10<sup>-8</sup> M. This shows that, in contrast to BALB/c progenitors, eosinophil precursors in this strain are as sensitive to inhibition by PGE<sub>2</sub> as those from BP-2 mice. Hence, the study of the BALB/c strain makes it possible to clearly distinguish between PGE<sub>2</sub> effects on progenitors and on precursors.

To define whether PGE<sub>2</sub> could act after bone-marrow cells had been exposed to rmIL-5, we established cultures in the presence of rmIL-5, and added PGE<sub>2</sub> as a single dose (to a final 10<sup>-7</sup> M concentration) to the cultures at various intervals. As shown in Table 3, while PGE<sub>2</sub> added at day 1 was as effective as PGE<sub>2</sub> present from the beginning of the culture in inhibiting responses to rmIL-5 from both BALB/c and BP-2 bone-marrow, addition of PGE<sub>2</sub> after culturing cells for 48 h in rmIL-5 alone was ineffective, even though these cultures were exposed to PGE<sub>2</sub> for 5 more days. These findings indicate that the target for PGE<sub>2</sub> is a relatively immature precursor, that probably becomes more resistant at later stages.

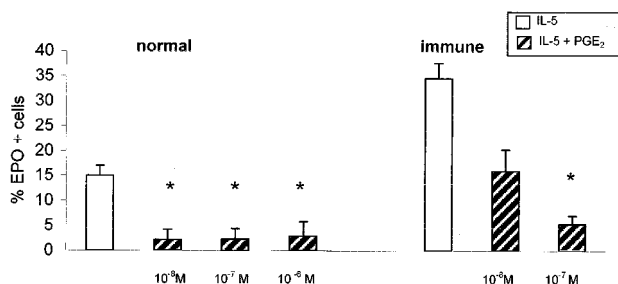
Inhibition of responses to rmIL-5 could be duplicated by cyclic AMP-elevating agents: in experiments with BP-2 bone-marrow, the per cent EPO+ was significantly decreased from 27 ± 5.6 (mean ± s.e.mean; data derived from six cultures) in the presence of rmIL-5 alone, to 3.1 ± 1.2 ( $P < 0.001$ ) when PGE<sub>2</sub> (10<sup>-7</sup> M) was added, and to 10.8 ± 2.8 ( $P < 0.01$ ) with addition of forskolin (3 × 10<sup>-5</sup> M). Addition of dbcAMP

(10<sup>-6</sup> M) to cultures from both BP-2 and BALB/c mice totally blocked responses to rmIL-5, and no intact EPO+ cells were present by day 7.

## Discussion

Several studies suggest that prostaglandins and cyclic AMP-elevating agents exert regulatory effects on haemopoiesis, both by modulating production of haemopoietic factors (Quill *et al.*, 1989; O'Reilly & Gamelli, 1990; Gamelli *et al.*, 1994; Bug *et al.*, 1998; Borger *et al.*, 1998; Kaminuma *et al.*, 1997), and by affecting the response to these factors (Gamelli *et al.*, 1998; Kozubik *et al.*, 1994; Piacibello *et al.*, 1991). Our findings provide further evidence that PGE<sub>2</sub> directly down-regulates the progenitor response to GM-CSF in murine bone-marrow. Experiments using several cyclic AMP-elevating agents yielded similar results, suggesting that these effects of PGE<sub>2</sub> involve cyclic AMP-dependent pathways. We have further examined whether the inhibitory effects of PGE<sub>2</sub> and dbcAMP, which are known to be modified by the state of maturation of the target cells (Piacibello *et al.*, 1991), by the presence of concurrent stimulatory signals (Visnjic *et al.*, 1995) and by target cell priming (Hallsworth *et al.*, 1996), would also be modified by allergen sensitization and challenge. Since similar effects were observed in bone-marrow cultures from ovalbumin-sensitized and challenged mice, and since PGE<sub>2</sub> (and other putative inhibitory prostaglandins) are generated both *in vivo* and in bone-marrow cultures (Gamelli *et al.*, 1994), the possible contribution of endogenous prostaglandin production to modulation of haemopoietic progenitors in murine bone-marrow must be taken into account when the effects of allergen sensitization and challenge on production of eosinopoietic cytokines (Minshall *et al.*, 1998), or on response to these cytokines (Gaspar Elsas *et al.*, 1997) are studied.

Our findings also allow for a comparison of the effects of PGE<sub>2</sub> and dbcAMP. Although both stimuli inhibited myeloid progenitors and eosinophil precursors, some differences were observed. As a rule, dbcAMP inhibited both targets more profoundly than PGE<sub>2</sub>. Furthermore, dbcAMP completely inhibited formation of eosinophil and granulocyte colonies, an effect that was not detectable in PGE<sub>2</sub>-treated cultures, even at very high prostaglandin concentrations. On the other hand, our findings differ from those of previous reports (Kurland *et al.*, 1977; Taetle & Koessler, 1980) in that: (a) we found no significant potentiation of colony formation by dbcGMP and (b) GM progenitors were not selectively inhibited by PGE<sub>2</sub> in our conditions. The reason for these discrepancies may be linked to the different experimental conditions, since these early studies used conditioned media, the effects of which are known to differ in important ways from those of pure, recombinant cytokines, due to the presence of a mixture of active factors in the former (Bagby, 1994).



**Figure 5** Effect of PGE<sub>2</sub> on IL-5-driven eosinophil differentiation in bone-marrow from BALB/c mice. Data are mean ± s.e.mean of the per cent EPO+ cells in liquid cultures established from naïve (normal) or ovalbumin-sensitized and challenged (immune) BALB/c mice in the presence of IL-5 (1 ng ml<sup>-1</sup>) alone or with PGE<sub>2</sub> at the indicated concentrations. Asterisks indicate significant differences relative to the respective rmIL-5 controls ( $P < 0.003$  in all cases). Data are derived from 9–10 experiments.

**Table 3** Effect of late addition of PGE<sub>2</sub> on IL-5-driven eosinophil differentiation in liquid culture

	BALB/c	P	BP-2	P
IL-5	29.3 ± 1.4		23 ± 1.5	
IL-5 + PGE <sub>2</sub> , day 0	9 ± 1.7	<0.001	6.6 ± 0.7	<0.001
IL-5 + PGE <sub>2</sub> , day 1	7 ± 2.7	<0.001	10.6 ± 0.6	<0.001
IL-5 + PGE <sub>2</sub> , day 2	23.6 ± 1.3	NS	23 ± 1.01	NS

Data are mean ± s.e.mean of the per cent EPO+ cells in liquid cultures established from ovalbumin-sensitized and challenged BALB/c and BP-2 mice, in the presence of 1 ng ml<sup>-1</sup> rmIL-5, without PGE<sub>2</sub> (IL-5) or with addition of PGE<sub>2</sub>, to final 10<sup>-7</sup> M concentration, at the indicated time points. Data are derived from three cultures for each point.

The inhibitory effects of PGE<sub>2</sub> varied among different inbred strains, and bone-marrow progenitors from BALB/c mice were totally resistant to PGE<sub>2</sub> over a large series of experiments. This argues against the effects of PGE<sub>2</sub> being due to nonspecific toxicity for bone-marrow progenitors. Furthermore, progenitors from BALB/c bone-marrow were inhibited by dbcAMP and by forskolin. This suggests that BALB/c mice have a defect in the progenitor response to PGE<sub>2</sub> that can be circumvented by cyclic AMP-elevating agents. Since isoproterenol did not inhibit colony formation in this strain, while it strongly suppressed progenitors from the C57BL/10 strain, which also responded to PGE<sub>2</sub>, it is possible that the defect in BALB/c progenitors affects receptor-cyclase coupling, although a decreased sensitivity of the cyclase to activating stimuli (such as forskolin) may also be present.

We have also analysed whether PGE<sub>2</sub> blocked lineage-specific responses to rmIL-5 in eosinophil precursors, an issue which has not been addressed in previous studies (Inman *et al.*, 1997; Sehmi *et al.*, 1997; Gaspar Elsas *et al.*, 1997). Our findings indicate that PGE<sub>2</sub> strongly inhibits IL-5-driven eosinophil differentiation. The availability of BALB/c mice, which resist inhibition of colony formation, made it possible to show that blockade of eosinophil precursor responses to rmIL-5 by PGE<sub>2</sub> can occur in the absence of any demonstrable effect on myeloid progenitors. Also, in the BP-2 strain, eosinophil progenitors were not inhibited by PGE<sub>2</sub> at 10<sup>-8</sup> M, but this prostaglandin concentration was inhibitory for eosinophil

precursor responses to rmIL-5. Finally, the examination of the frequency of the different types of myeloid colonies provided no evidence of a significant effect on the eosinophil lineage at the progenitor level. In contrast, the analysis of the lineage-specific responses of eosinophil precursors to rmIL-5 revealed a significant effect of PGE<sub>2</sub> at concentrations lower than those that inhibited the responses of progenitors of most mouse strains to GM-CSF. Again, inhibition of precursor responses did not seem to involve nonspecific toxicity, because: (a) addition of PGE<sub>2</sub> after 48 h of the beginning of the culture did not inhibit responses to rmIL-5, and (b) cultures established in the presence of rmIL-5 and PGE<sub>2</sub> did show a progressive increase in EPO+ cell numbers, showing that the conditions in the culture were permissive for the survival and differentiation of these cells, even though the magnitude of the plateau response was strongly reduced.

Taken together, our findings indicate that GM-CSF-responsive myeloid progenitors and IL-5-stimulated eosinophil precursors, which are known to differ in proliferative potential and colony-forming ability (Bagby, 1994) constitute separate targets for down-modulation by PGE<sub>2</sub>.

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