

PROSTAGLANDIN (PG) D<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> are anti-proliferative eicosanoids. We investigated the production of PGD<sub>2</sub> by murine bone marrow-derived mast cells (BMMC) taking into consideration metabolism of PGD<sub>2</sub> to PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub>. PG-metabolites were quantified by high performance liquid chromatography (HPLC) combined with radioimmunoassay (RIA). Stimulated with calcium ionophore A23187 BMMC released eight-fold more PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> than PGD<sub>2</sub>. Conversion of endogenously produced PGD<sub>2</sub> to PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> proceeded rapidly in contrast to metabolism of exogenously added PGD<sub>2</sub>. The antiproliferative potency of these prostaglandins is demonstrated *in vitro*. We conclude that determination of PGD<sub>2</sub> production by mast cells must take into consideration rapid conversion to active derivatives, which may play a significant role in growth regulation.

**Key words:** Prostaglandin D<sub>2</sub>, Prostaglandin J<sub>2</sub>,  $\Delta^{12}$ -Prostaglandin J<sub>2</sub>, Cyclopentenone, Anti-proliferative activity, Mast cell

## Release of prostaglandin D<sub>2</sub> by murine mast cells: importance of metabolite formation for antiproliferative activity

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## Introduction

Prostaglandin (PG) D<sub>2</sub> and its metabolites PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> are potent antiproliferative eicosanoids.<sup>1</sup> The concentrations of PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> required for regulation of cell proliferation *in vitro* are in the micromolar range.<sup>1</sup> The high molar activity suggests that these lipid mediators may play a role in the regulation of cell proliferation *in vivo* and that, on the other hand, this principle may be used in anti-tumour therapy. In fact, the related chemically stabilized prostanoid  $\Delta^7$ -PGA<sub>1</sub> has been proposed for clinical studies.<sup>2</sup> PGD<sub>2</sub> may also represent a component in natural anti-neoplastic defence. Mast cells are regarded as major sources of endogenous PGD<sub>2</sub> production. Murine bone marrow-derived mast cells (BMMC) have been reported to produce 15–120 pmol PGD<sub>2</sub> per million cells during 10–30 min of incubation following different kinds of stimulation.<sup>3,4</sup> However, these apparently low production rates of PGD<sub>2</sub> are unlikely to exert major biological effects *in vivo*.

We hypothesized that the reported PGD<sub>2</sub> production rates underestimate biosynthesis of this mediator, because PGD<sub>2</sub> can be converted to other active metabolites including antiproliferative PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub>. These cyclopentenone prostanoids have been shown to occur in aqueous media, blood plasma and urine.<sup>1</sup>

In the present study PGD<sub>2</sub> and its metabolites were detected and quantified in supernatants from murine mast cells by combined use of high performance liquid chromatography (HPLC) and radioimmunoassay (RIA) analysis. The antiproliferative potencies of PGD<sub>2</sub> and its derivatives PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> were evaluated testing the effect of these PGs on [<sup>3</sup>H]-thymidine incorporation into human myelocytic (HL-60), monocytoid (THP-1, U937) and lymphoid (Burkitt, Raji) cell lines and on MIT-conversion by murine bone marrow-derived mast cells (BMMC) and IL-3-dependent and autonomous mast cell lines.

## Materials and Methods

### Chemicals

Unlabelled PGD<sub>2</sub>, PGJ<sub>2</sub>,  $\Delta^{12}$ -PGJ<sub>2</sub>, LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> were from Paesel & Lorei, Frankfurt, Germany, 5,6,8,9,11,12,14,15-[<sup>3</sup>H]-PGD<sub>2</sub> (3,7 MBq/ml), 14,15-[<sup>3</sup>H]-LTC<sub>4</sub>, -LTD<sub>4</sub>, -LTE<sub>4</sub> (925 kBq/ml), [1-<sup>14</sup>C]-arachidonic acid (AA) (1,85 MBq/qml), PGD<sub>2</sub>-RIA-kit, and [methyl-<sup>3</sup>H]-thymidine ([<sup>3</sup>H]-T) (37 MBq/ml) were from Amersham-Buchler, Braunschweig, Germany. Calcium-ionophore A23187, indomethacin, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (HIMP) and gelatine from porcine skin (300 bloom) were from Sigma, St Louis, MO, USA. 3-(4,5-dimethyl-2-thiazolyl)-

2,5-diphenyl-2H-tetrazolium-bromid (MIT) was from Serva, Heidelberg, Germany. Fetal calf serum (FCS) and RPMI 1640 were from Biochrom, Berlin, Germany. The other chemicals used were purchased from Merck, Darmstadt, Germany and were at least of analytical grade.

### Cells and cell lines

Primary polyclonal BMMC were generated in cultures of murine (BALB/c) bone marrow cells supplemented with pokeweed mitogen-activated spleen cell-conditioned medium (SCM) as previously described.<sup>5</sup> Briefly, murine bone marrow cells ( $1 \times 10^5$ /ml) were grown in RPMI-1640 medium supplemented with 20% FCS, 20% SCM, 2 mM L-glutamine, 100 U/ml penicillin-streptomycin and  $10^{-4}$  M  $\alpha$ -thioglycerol. When propagating non-adherent bone marrow cells in this culture medium with weekly refeeding, homogenous populations of mast cells could be obtained within a period of 3 to 8 weeks. Mast cells used for analysis of eicosanoid production were 21–55 days *ex vivo* ( $33 \pm 10$  days; mean  $\pm$  standard deviation (SD)). IL-3-dependent long-term cultured mast cell lines were derived from BMMC.<sup>5</sup> A growth factor independent malignant autonomous mast cell line was derived from an IL-3-dependent long term cultured mast cell line.<sup>6</sup>

A human myeloid leukaemic cell line (HL-60)<sup>7</sup> was cultured in RPMI 1640 containing 15% FCS and 100 U/ml penicillin-streptomycin. Human monocytic (THP-1)<sup>8</sup> and histiocytic (U937)<sup>9</sup> cell lines were cultured in RPMI 1640 with 10% FCS and 100 U/ml penicillin-streptomycin. Human Burkitt<sup>10</sup> and Raji<sup>11</sup> lymphoma cell lines were cultured in RPMI 1640 containing 10% FCS and 100 U/ml penicillin-streptomycin. HL-60-, THP-1-, U937-, Burkitt and Raji lymphoma cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were cultured at 37°C under humidified atmosphere with 5% (mast cells) or 10% CO<sub>2</sub>.

### Determination of prostaglandin and leukotriene production

For analysis of radioactive AA-metabolites  $5 \times 10^7$  of BMMC were prelabelled with [<sup>14</sup>C]-AA (18.5 kBq/ml) for 48 h. Cells were washed twice and resuspended in PBS ( $10^7$  cells/ml) containing gelatine (0.5 mg/ml) or in culture medium (RPMI 1640 with 20% FCS). After a 5-min period of preincubation, cells were incubated with or without calcium ionophore A23187 (0.2  $\mu$ M) at 37°C. Incubation was stopped by centrifugation (10 min at 4°C and  $600 \times g$ ). For deproteinization supernatant and pellet were separated and added to 8 volumes of 90% aqueous methanol containing 0.5 mM

EDTA and 1 mM HIMP, pH 7.4. HIMP was used for stabilization of lipid mediators. The suspensions were stored at  $-40^\circ\text{C}$  for at least 12 h and then centrifuged (20 min at  $-10^\circ\text{C}$  and  $9000 \times g$ ). Supernatants were evaporated to dryness in a centrifuge under low pressure (Speed Vac, Savant, Farmingdale, NY, USA) and redissolved in 30% aqueous methanol containing trace amounts of unlabelled PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub>.

[<sup>14</sup>C]-AA-metabolites were separated by HPLC performed on a C18 Hypersil column ( $4.6 \times 250$  mm, 5  $\mu$ m particles; Shandon, Runkorn, UK) with a C18 precolumn (Waters, Milford, MA, USA). The mobile phase consisted of methanol, water, acetic acid (65:35:0.1 by volume), pH 4.0 adjusted with ammonium hydroxide. The flow rate was 0.4 ml/min. After a 45 min period the methanol concentration in the solvent was gradually increased to 100%. Metabolites were detected by liquid scintillation counting (HPLC radioactivity monitor LB 507 A; Berthold, Wildbad, Germany). [<sup>14</sup>C]-AA-metabolites (PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub>) were identified by comigration with unlabelled standards as detected at 220 nm (Spectrophotometer, Waters, Milford, MA, USA).

Analysis of prostaglandin and leukotriene production by unlabelled mast cells was performed by sequential use of HPLC and RIA. BMMC were washed twice and resuspended in PBS (0.5 mg/ml gelatine) at a concentration of  $10^6$  cells/ml. Incubation and deproteinization were performed as described above. Samples were redissolved in 30% aqueous methanol containing trace amounts of [<sup>3</sup>H]-labelled PGD<sub>2</sub> or LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> standards.

The mobile phase of HPLC for routine prostaglandin analysis consisted of methanol, water, acetic acid (65:35:0.1 by volume), pH 4.0 adjusted with ammonium hydroxide (flow rate: 0.4 ml/min). For confirmation of the identity of endogenously produced PGD<sub>2</sub> metabolites, additional solvent systems were used: acetonitrile, water, acetic acid (40:60:0.1 by volume), flow rate: 0.4 ml/min and acetonitrile, water and acetic acid (50:50:0.1 by volume), flow rate: 0.5 ml/min. The mobile phase for routine leukotriene analysis consisted of methanol, water, acetic acid (65:35:0.1 by volume), pH 5.6 adjusted with ammonium hydroxide (flow rate: 1.0 ml/min). HPLC-fractions were collected in 0.5-min intervals. An aliquot of these fractions was counted for tritium radioactivity to determine retention time of [<sup>3</sup>H]-standards. Another aliquot was stored at  $-20^\circ\text{C}$  under argon for further analysis by RIA.

The commercial [<sup>3</sup>H]-PGD<sub>2</sub>-RIA (Amersham Buchler, Braunschweig, Germany) of HPLC-fractions was performed as described by the manufacturer. We determined cross-reactivities of PGJ<sub>2</sub>- and  $\Delta^{12}$ -PGJ<sub>2</sub> to be 7% and 24%, respectively. The [<sup>3</sup>H]-cysteinyl leukotriene-RIA was performed as described earlier.<sup>12</sup>

### Determination of metabolism of [<sup>3</sup>H]-PGD<sub>2</sub>

Metabolism of [<sup>3</sup>H]-PGD<sub>2</sub> to [<sup>3</sup>H]-PGJ<sub>2</sub> and [<sup>3</sup>H]- $\Delta^{12}$ -PGJ<sub>2</sub> was determined by HPLC following incubation of [<sup>3</sup>H]-PGD<sub>2</sub> (3.7 kBq/ml) for 0 min to 24 h in phosphate buffered saline (PBS) or in culture medium. Controls in PBS were performed with and without BMMC (10<sup>6</sup> cells/ml) and with and without gelatine (0.5 mg/ml). Preparation of samples for HPLC was performed as described for analysis of endogenous PG-production including the deproteinization procedure. Retention times of [<sup>3</sup>H]-PGD<sub>2</sub> and its metabolites were established as described for analysis of [<sup>14</sup>C]-AA metabolites.

### Proliferation assays

Cells were incubated for 24 h with or without PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> at concentrations of 10 nM to 0.1 mM. [<sup>3</sup>H]-T uptake into cells was used as a parameter of DNA-replication and cell proliferation. After 24 h of incubation in the presence of [<sup>3</sup>H]-T in microtitre plates (37 kBq/well) cells were collected on glassfibre filters by a cell harvester (Canberra-Packard, Dreieich, Germany) and radioactivity associated with the washed and dried filters was quantified with a Matrix 96 counter (Canberra-Packard, Dreieich, Germany) as  $\beta$ -counts. Alternatively, a colorimetric proliferation assay, the MTT-test, was used as described.<sup>5</sup> Briefly, cultured cells in microtitre plates were incubated for 4 h with MTT. Incubation was stopped with acidified isopropanol. Optical density (OD) was detected in a ELISA-reader (SLT, Salzburg, Austria) at a test wave length of 550 nm and a reference wave length of 690 nm. Results are shown as OD 550–690 values.

## Results

Mast cells release PGD<sub>2</sub> that is rapidly converted to PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub>

Following labelling with [<sup>14</sup>C]-AA and stimulation with calcium ionophore A23187 BMMC released [<sup>14</sup>C]-AA and small amounts of its metabolites co-migrating on HPLC with LTC<sub>4</sub>, PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> (not shown). In addition a number of unidentified metabolites were formed.

Release of unlabelled PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> by BMMC was quantified by combined use of HPLC and RIA. PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> were detected in samples stimulated with A23187 (0.2  $\mu$ M) for 30 min (Fig. 1). The total concentration of these mediators amounted to  $153.6 \pm 78.4$  pmol/10<sup>6</sup> cells ( $n = 4$ ) (Fig. 2). The relative quantities of PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> were  $11 \pm 8\%$ ,  $53 \pm 18\%$  and  $36 \pm 17\%$  ( $n = 4$ , mean  $\pm$  SD), respectively (Figs. 1 and 2). The A23187-stimulated release of PGD<sub>2</sub>, PGJ<sub>2</sub> plus  $\Delta^{12}$ -PGJ<sub>2</sub> was  $169\% \pm 14\%$  of the unstimulated samples ( $n = 3$ , mean  $\pm$  SD) (Fig. 2).

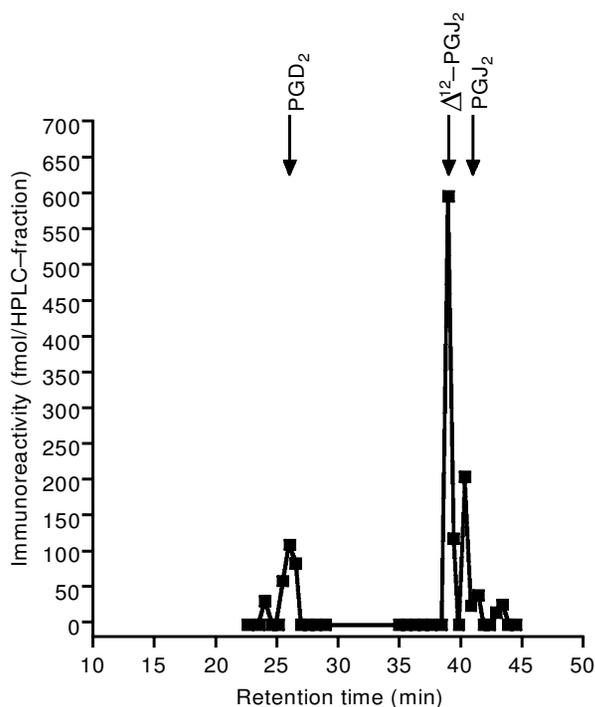


FIG. 1. PGD<sub>2</sub> and its metabolites detected by [<sup>3</sup>H]-PGD<sub>2</sub>-RIA in HPLC fractions of a supernatant of BMMC. BMMC were stimulated with calcium ionophore A23187 (0.2  $\mu$ M). Supernatants were separated by HPLC and HPLC-fractions were analysed by RIA. Retention times of authentic PGD<sub>2</sub>, PGJ<sub>2</sub> and  $\Delta^{12}$ -PGJ<sub>2</sub> standards are indicated by arrows.

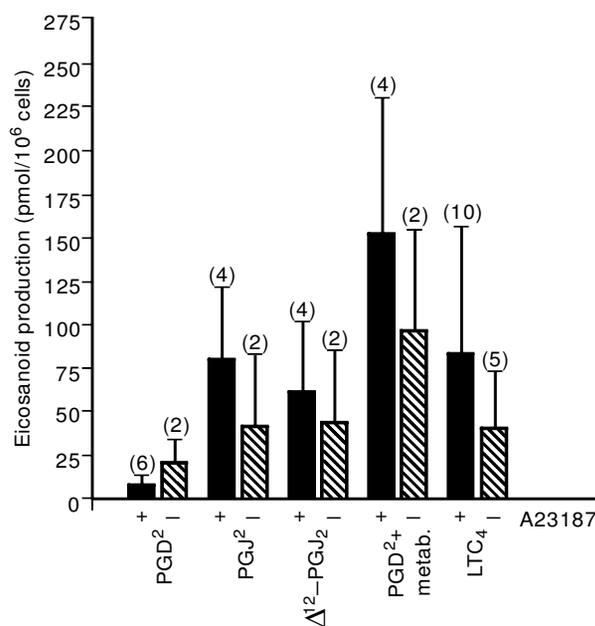


FIG. 2. Endogenous production of PGD<sub>2</sub>, PGJ<sub>2</sub>,  $\Delta^{12}$ -PGJ<sub>2</sub> and of total of these prostaglandins (PGD<sub>2</sub> + metab.) and of LTC<sub>4</sub>. BMMC were suspended in PBS with (+) or without (-) calcium ionophore A23187 (0.2  $\mu$ M) for 30 min at 37°C. Results are given as mean  $\pm$  SD or range of two to 10 independent experiments (number in parenthesis).

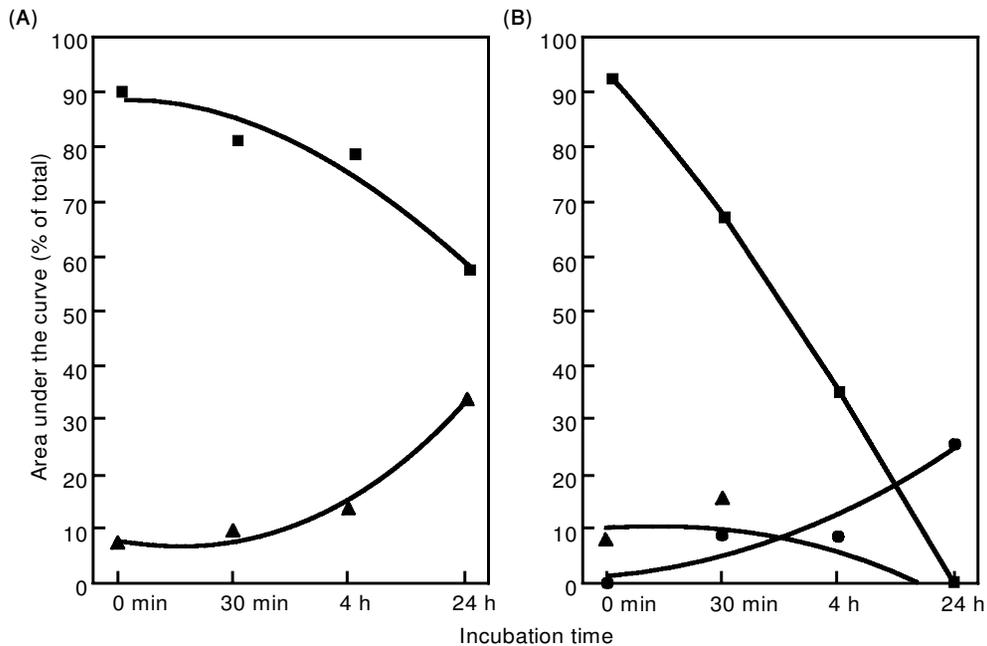


FIG. 3. Metabolism of [<sup>3</sup>H]-PGD<sub>2</sub> in PBS (A) and culture medium containing FCS (B). After incubation of [<sup>3</sup>H]-PGD<sub>2</sub> in PBS or culture medium, samples were processed as described in the Methods section. [<sup>3</sup>H]-PGD<sub>2</sub> and its metabolites were detected by continuous radiodetection in the HPLC eluate. [<sup>3</sup>H]-PGD<sub>2</sub> (■), [<sup>3</sup>H]-PGJ<sub>2</sub> (▲) and [<sup>3</sup>H]-Δ<sup>12</sup>-PGJ<sub>2</sub> (●) were identified by co-migration with authentic unlabelled standards. [<sup>3</sup>H]-Δ<sup>12</sup>-PGJ<sub>2</sub> could only be detected in culture medium containing FCS.

The identity of PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> released in the supernatant by BMMC was confirmed by co-migration with authentic [<sup>3</sup>H]-labelled PGD<sub>2</sub> and authentic unlabelled PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> with varying mobile phases and flow rates as described in the Methods section (data not shown).

PGD<sub>2</sub> is spontaneously metabolized to PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub>

PGD<sub>2</sub> was metabolized to PGJ<sub>2</sub> in PBS. Further metabolism to Δ<sup>12</sup>-PGJ<sub>2</sub> occurred in culture medium containing FCS (Fig. 3). Following 30 min of incubation

in PBS or culture medium 19% or 33% of [<sup>3</sup>H]-PGD<sub>2</sub> were metabolized, respectively. The half-life of PGD<sub>2</sub> was more than 10 h in PBS and 3 h in culture medium. Metabolism of extracellular PGD<sub>2</sub> was not altered in the presence of 10<sup>6</sup> BMMC per ml. Gelatine (0.5 mg/ml) had also no effect on PGD<sub>2</sub> metabolism. The procedures of sample preparation had no influence on recovery or metabolism of PGD<sub>2</sub>.

BMMC produce large amounts of LTC<sub>4</sub>

Following 30 min of incubation, BMMC produced 85.4 ± 74.0 pmol LTC<sub>4</sub>/10<sup>6</sup> cells (n = 10) and 42.1 ±

**Table 1.** Concentrations of PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> required for 50% inhibition of cell proliferation (IC<sub>50</sub>) in selected cell lines (n = 4)

Cell culture	IC <sub>50</sub> of		
	PGD <sub>2</sub>	PGJ <sub>2</sub>	Δ <sup>12</sup> -PGJ <sub>2</sub>
HL-60-myelocytic cell line (human)	11 μM	11.5 μM	12 μM
Primary bone marrow-derived mast cells (BMMC) (murine)	10 μM	25 μM	20 μM
IL-3-dependent mast cell line (murine)	4 μM	9 μM	5 μM
Autonomous mast cell line (murine)	4 μM	11 μM	6 μM
THP-1 monocytic cell line (human)	50 μM	50 μM	30 μM
U937 histiocytic cell line (human)	70 μM	80 μM	40 μM
Burkitt lymphoma cell line (human)	2.5 μM	3.5 μM	5 μM
Raji lymphoma cell line (human)	7 μM	7 μM	9 μM

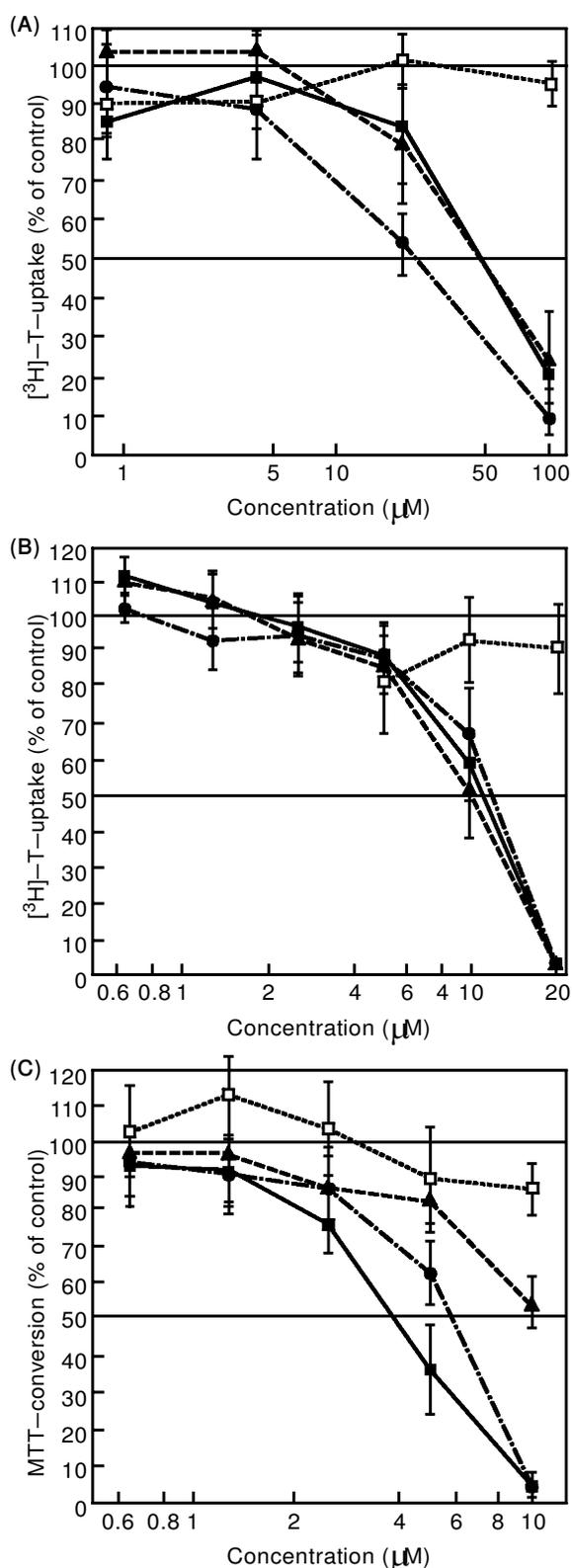


FIG. 4. Modulation of cell proliferation by PGD<sub>2</sub> and by its metabolites. Results obtained in a THP-1 monocytic cell line (human) (A), a HL-60 myeloid leukemic cell line (human) (B) and a murine autonomous mast cell line (C) are demonstrated. Results are expressed in percentage of control of [<sup>3</sup>H]-T-uptake or MTT-conversion. PGD<sub>2</sub> (■), PGJ<sub>2</sub> (▲) and Δ<sup>12</sup>-PGJ<sub>2</sub> (●) show distinct antiproliferative activity. Indomethacin (□) had no significant effect on cell proliferation. Experiments were performed in quadruplicates (mean ± SD).

33.8 pmol LTC<sub>4</sub>/10<sup>6</sup> cells ( $n = 5$ ; mean ± SD) with and without calcium ionophore A23187 (0.2 μM), respectively. LTC<sub>4</sub> was not metabolized to LTD<sub>4</sub> or LTE<sub>4</sub> under the conditions used.

#### PGD<sub>2</sub> and its derivatives PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> exert strong antiproliferative activity

PGD<sub>2</sub> and its metabolites inhibited cell proliferation in all *in vitro* models tested (Table 1, Fig. 4). The IC<sub>50</sub> values varied between 2.5 and 80 μM in different cell types. PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> were equally effective. At concentrations below 0.5 μM, no significant effect on cell proliferation was observed. Indomethacin (0.1 mM to 10 nM) had no influence on the proliferation of BMMC, TPH-1, U937 or HL-60 cells (Fig. 4). PGD<sub>2</sub> was rapidly metabolized in culture medium to PGJ<sub>2</sub>, Δ<sup>12</sup>-PGJ<sub>2</sub> and other not identified metabolites or conjugates.

#### Discussion

Our data demonstrate that murine BMMC release PGD<sub>2</sub> and LTC<sub>4</sub>, potent eicosanoid mediators. PGD<sub>2</sub> undergoes immediate metabolism to PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> during production by BMMC (Fig. 1). This rapid metabolism of PGD<sub>2</sub> is not explained by degradation in extracellular fluid, because the half-life of PGD<sub>2</sub> is more than 10 h in PBS and 3 h in culture medium containing FCS. Mast cells do not contribute to conversion of extracellular PGD<sub>2</sub>.

The total of PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> produced by stimulated mast cells is about eight-fold the amount of PGD<sub>2</sub> alone (Figs 1 and 2). Separation of PGD<sub>2</sub>-metabolites is required prior to RIA, because considerable differences exist in the immunoreactivity of the various PGD<sub>2</sub> metabolites corresponding to very different cross-reactivity profiles in radio-immunologic analysis. Direct radio-immunologic analysis<sup>3</sup> may thus result in gross underestimation of the PGD<sub>2</sub> production and of the PGD<sub>2</sub> mediated antiproliferative potential of BMMC. Stimulation of BMMC with A23187 significantly increased the release of PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> in total. This is largely due to an increased release of the latter two metabolites (Fig. 2).

A number of peaks detected in the HPLC chromatogram of supernatant or pellet of [<sup>14</sup>C]-AA-labelled BMMC have not yet been identified. Some of them may represent further products derived from PGD<sub>2</sub>.<sup>1,13-15</sup> Identification of these metabolites requires more efficient procedures to label BMMC with [<sup>14</sup>C]-AA. The respective experiments are under way.

In contrast to PGD<sub>2</sub> LTC<sub>4</sub> remained stable under the conditions investigated, i.e. in serum-free medium. The amounts of LTC<sub>4</sub> produced in our model of murine BMMC accord with data shown by Razin *et al.*<sup>3</sup> indicating that BMMC represent a reproducible model for analysis of eicosanoid production.

The metabolites PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> were about equipotent to PGD<sub>2</sub> in their anti-proliferative activity on myelocytic, mastocytic, monocytic/histiocytic and lymphocytic cells (Table 1, Fig. 4). PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> had distinct antiproliferative effects with IC<sub>50</sub> values ranging from 2.5 to 80 μM. The metabolism of PGD<sub>2</sub> in culture medium (Fig. 3) and the fact that there were no significant differences in the antiproliferative effects of PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> in the proliferation assays using incubation times of 24 h (Table 1, Fig. 4) are both in line with the suggestion of Narumiya and Fukushima<sup>1,15</sup>, that Δ<sup>12</sup>-PGJ<sub>2</sub> is the active metabolite of PGD<sub>2</sub> and that PGD<sub>2</sub> may exert no growth inhibition by itself.

The mechanism of action of Δ<sup>12</sup>-PGJ<sub>2</sub> is not fully understood. There are no surface receptors. The lipid mediator is actively transported into cells through a temperature-dependent transporter on plasma membranes. Δ<sup>12</sup>-PGJ<sub>2</sub> accumulates in the nuclei, where it binds to thiol groups of nuclear proteins.<sup>16</sup>

Glutathione depletion enhances antiproliferative activity of PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub>.<sup>14</sup> Recently it has been shown that intracellular glutathione modulates induction of apoptosis by Δ<sup>12</sup>-PGJ<sub>2</sub>.<sup>17</sup>

PGD<sub>2</sub> and its metabolites exerted a more distinct antiproliferative effect in lymphocytic, myelocytic and mast cells than in monocytic or histiocytic cells. Primary murine BMMC were more resistant to growth inhibition by PGD<sub>2</sub>, PGJ<sub>2</sub> or Δ<sup>12</sup>-PGJ<sub>2</sub> than IL-3-dependent and autonomous mast cell lines (Table 1). Indomethacin had no effect on proliferation of BMMC, U937 or HL-60 cells in the proliferation assays used.

We conclude that measurement of PGD<sub>2</sub> disregarding its conversion to metabolites may grossly underestimate PGD<sub>2</sub> production and effects related to these mediators. Our results suggest that the total of PGD<sub>2</sub>, PGJ<sub>2</sub> and Δ<sup>12</sup>-PGJ<sub>2</sub> produced by stimulated mast cells may be expected to exert significant antiproliferative activity. Production of antiproliferative PGD<sub>2</sub> metabolites by BMMC *in vitro* raises the possibility that mast cells exert an anti-proliferative and anti-neoplastic activity by the release of anti-proliferative PGD<sub>2</sub> metabolites *in vivo*.

## References

1. Fukushima M. Prostaglandin J<sub>2</sub>-anti-tumour and anti-viral activities and the mechanisms involved. *Eicosanoids* 1990; **3**: 189–199.
2. Sasaki H, Fukushima M. Prostaglandins in the treatment of cancer. *Anticancer Drugs* 1994; **5**: 131–138.
3. Razin E, Mencia-Huerta JM, Lewis RA, Corey EJ, Austen KE. Generation of leukotrien C<sub>4</sub> from a subclass of mast cells differentiated in vitro from mouse bone marrow. *Proc Natl Acad Sci USA* 1982; **79**: 4665–4667.
4. Murakami M, Penrose JF, Urade Y, Austen KE, Arm JP. Interleukin 4 suppresses c-kit ligand-induced expression of cytosolic phospholipase A<sub>2</sub> and prostaglandin endoperoxide synthase 2 and their roles in separate pathways of eicosanoid synthesis in mouse bone marrow-derived mast cells. *Proc Natl Acad Sci USA* 1995; **92**: 6107–6111.
5. Hältner L, Moeller J, Schmitt E, et al. Thiol-sensitive mast cell lines derived from mouse bone marrow respond to a mast cell growth-enhancing activity different from both IL-3 and IL-4. *J Immunol* 1989; **142**: 3440–3446.
6. Hältner L, Moeller J, Dörmer P. Reproducible generation of autonomous malignant sublines from non-tumorigenic murine interleukin 3-dependent mast cell lines. *Blut* 1986; **53**: 451–455.
7. Collins SJ, Gallo RC, Gallagher RE. Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. *Nature* 1977; **270**: 347–349.
8. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer* 1980; **26**: 171–176.
9. Ralph P, Moore MAS, Nilsson K. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. *J Exp Med* 1976; **143**: 1528–1533.
10. Calender A, Billaud M, Aubry J-P, Banchereau J, Vuillaume M, Lenoir GM. Epstein-Barr virus (EBV) induces expression of B-cell activation markers on in vitro infection of EBV-negative B-lymphoma cells. *Proc Natl Acad Sci USA* 1987; **84**: 8060–8064.
11. Epstein MA, Achong BG, Barr YM, Zajac B, Henle G, Henle W. Morphological and virological investigations on cultured burkitt tumor lymphoblasts (strain Raji). *J Natl Cancer Inst* 1966; **37**: 547–559.
12. Denzlinger C, Haberl C, Wilmanns W. Cysteinyl leukotriene production in anaphylactic reactions. *Int Arch Allergy Immunol* 1995; **108**: 158–164.
13. Atsmon J, Sweetman BJ, Baertschi SW, Harris TM, Roberts II LJ. Formation of thiol conjugates of 9-deoxy-Δ<sup>9</sup>, Δ<sup>12</sup> (E)-prostaglandin D<sub>2</sub> and Δ<sup>12</sup> (E)-prostaglandin D<sub>2</sub>. *Biochemistry* 1990; **29**: 3760–3765.
14. Atsmon J, Freeman ML, Meredith MJ, Sweetman BJ, Roberts II LJ. Conjugation of 9-deoxy-Δ<sup>9</sup>, Δ<sup>12</sup> (E)-prostaglandin D<sub>2</sub> with intracellular glutathione and enhancement of its antiproliferative activity by glutathione depletion. *Cancer Research* 1990; **50**: 1879–1885.
15. Narumiya S, Fukushima M. Δ<sup>12</sup>-Prostaglandin J<sub>2</sub>, an ultimate metabolite of prostaglandin D<sub>2</sub> exerting cell growth inhibition. *Biochem Biophys Res Commun* 1985; **127**: 739–745.
16. Negishi M, Koizumi T, Ichikawa A. Biological actions of Δ<sup>12</sup>-prostaglandin J<sub>2</sub>. *J Lipid Mediators Cell Signalling* 1995; **12**: 443–448.
17. Kim HS, Lee J-H, Kim I-K. Intracellular glutathione level modulates the induction of apoptosis by Δ<sup>12</sup>-prostaglandin J<sub>2</sub>. *Prostaglandins* 1996; **51**: 413–425.

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