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Prostanoid release by macrophages at a distance from an inflammatory site

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Summary: During the development of an acute inflammatory reaction induced in the rat pleural cavity by dextran, calcium pyrophosphate, saline or phosphate buffered saline, macrophages present at a distant site (peritoneal cavity) display an increased capacity to release prostanoids: prostaglandins, prostacyclin and thromboxane. Enhanced levels of 6-keto-$PGF_{1\alpha}$ were observed both in peritoneal lavages (experiments *in vivo*) and in macrophage supernatants after 24-h culture (experiments *in vitro*). TXB_2 levels were mainly increased in peritoneal lavages and PGE_2 in culture supernatants. *In vivo*, levels of prostanoids in the peritoneal cavity reached a maximum 24 h after the induction of pleurisy whatever the injected substance. *In vitro*, amounts of arachidonic acid metabolites were highest in supernatants of cultured peritoneal macrophages harvested 72 h after the pleural injection of dextran or CaPP. These results show that the regulation of macrophage functions is closely related to prostanoid production, especially the release of PGE_2 and PGI_2.

Keywords: pleurisy, macrophages, prostanoids

The release of prostanoids (PGE_2, $PGF_{2\alpha}$, PGI_2, TXA_2) has been demonstrated at the site of various types of acute or chronic inflammatory processes. Some authors have described the kinetics of prostanoid release in pleural exudates induced in the rat by carrageenan (Di Rosa *et al.* 1971; Higgs & Salmon 1979; Katori *et al.* 1980; Tissot *et al.* 1984a; Velo *et al.* 1973; White *et al.* 1985) or calcium pyrophosphate (CaPP) (Capasso *et al.* 1975; Tissot *et al.* 1984b; Willoughby *et al.* 1975). Similar observations have been made in rats with the sponge model (Bird *et al.* 1986; Higgs & Salmon 1979), the air pouch model (Ohuchi *et al.* 1982; Sedgwick & Lees 1986), in synovial joints of chronic arthritic rabbits (Henderson *et al.* 1985) and in inflamed rabbit cornea (Bazan *et al.* 1985).

In addition to this local production of prostanoids, we have demonstrated that certain properties of cells collected at a distance from the site of injury are modified by inflammatory stimuli: production of increased amounts of interleukin-1-like material by peritoneal macrophages (Bird *et al.* 1985c; Yao *et al.* 1984), modulation of their oxidative metabolism as evaluated by chemiluminescence and superoxide release (Bird *et al.* 1985a, 1985b), modulation of mitogenic and cytostatic activity of macro-

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phages (Giroud *et al.* 1981; 1983), modulation of the proliferative response to PHA of lymph node and spleen cells *in vitro*. (Yao *et al.* 1984) and modifications of immune mechanisms which play a role in defence against infection and neoplasia observed in the liver, spleen and peritoneum (Florentin *et al.* 1985). Possible mediators for these systemic effects include prostanoids.

The aim of this work was to investigate *in vivo* and *in vitro* the release of prostanoids by peritoneal macrophages harvested from rats undergoing a pleural inflammatory reaction. For this purpose the production of PGE_2 and $PGF_{2\alpha}$, prostacyclin and thromboxane were measured in peritoneal lavages collected from 4 h to 5 days after the induction of pleurisy by CaPP, saline or phosphate buffered saline (PBS) and 24 h after the pleural injection of dextran; the same prostanoids were measured in 24-h culture supernatants of peritoneal macrophages harvested 1–5 days after the onset of pleurisy induced by CaPP crystals, dextran, PBS or saline.

Materials and methods

Inflammatory reactions

Sterile pleurisy was induced in male Sprague–Dawley rats weighing 180–200g, by injecting 1 ml of either a 1% suspension of anhydrous calcium pyrophosphate microcrystals (Willoughby *et al.* 1975), a 6% solution of 40 000 mol. wt. dextran (Pelletier *et al.* 1978), saline (pH 5.9) or phosphate buffered saline (pH 7.4) into the pleural cavity. Untreated rats served as controls.

Collection of peritoneal fluids (in-vivo assays)

Rats with pleurisy and controls were injected intraperitoneally (i.p.) with 10 ml of cold, pyrogen-free saline. Peritoneal washing fluids were collected in polypropylene tubes and acetylsalicylic acid was immediately added at a final concentration of 2×10^{-4} mol $\times l^{-1}$. Cells were removed by centrifugation (1200 *g*, 5 min) and supernatants were stored at −20°C until prostanoid assays.

Culture of peritoneal macrophages (in-vitro assays)

Peritoneal cells were harvested under sterile conditions from rats with pleurisy and from control rats by injecting i.p. 2 × 10 ml of 199 culture medium supplemented with 20% heat-inactivated new-born calf serum, antibiotics and heparin (Pelletier *et al.* 1978). Peritoneal washing fluids were centrifuged; cells were then resuspended in complete culture medium, distributed in triplicate in Leighton tubes at a concentration of 2.5×10^6 cells/1.5 ml/tube and were incubated for 3 h at 37°C to allow the macrophages to adhere to the glass coverslips. The non-adherent cells were eliminated and macrophages were further incubated with 1.5 ml of fresh complete culture medium for 24 h at 37°C. Culture supernatants were then collected, centrifuged and stored at −20°C until prostanoid assays.

Prostanoid assays

PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 (stable metabolites of PGI_2 and TXA_2 respectively) were measured using radioimmunoassay in the supernatants of washing fluids of the peritoneal cavity or in supernatants of macrophages cultures, diluted with phosphate buffer. Aliquots of various dilutions of the supernatants were assayed as previously described (Bird *et al.* 1985a; 1985b; Tissot *et al.* 1984a; 1984b). PGE_2 and $PGF_{2\alpha}$ were assayed using anti-sera from Institut Pasteur Production, ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ from NEN (France), and unlabelled PGE_2 and $PGF_{2\alpha}$ from Sigma (USA). 6-keto-$PGF_{1\alpha}$ and TXB_2 were determined using a commercial kit (NEN, France).

Statistical analysis

The data were analysed for statistical significance using Student's *t* test. The results

represent the mean ± s.e.m. of n experiments, where n is the number of rats at each time.

Results

In-vivo prostanoid release by peritoneal cells

The concentrations of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 were measured in the peritoneal washing fluids collected from 4 h to 5 days after intrapleural injection of CaPP suspension or saline.

The results presented in Fig. 1a show that the liberation of the four prostanoids was significantly augmented 24 h after CaPP-induced pleurisy as compared to untreated controls. This stimulatory effect was greatest on 6-keto-$PGF_{1\alpha}$ and TXB_2 release: their levels were increased approximately three-fold. Moreover, the increase in 6-keto-$PGF_{1\alpha}$ was detected as early as 4 h after pleurisy induction. Prostanoid concentrations returned to values of uninjected controls from day 3 onwards.

As seen in Fig. 1b, intrapleural injection of saline induced changes in prostanoid concentrations in peritoneal fluids of a magnitude similar to those resulting from CaPP injection. However, the early augmentation at 4 h in 6-keto-$PGF_{1\alpha}$ levels was not observed.

Several types of insoluble and soluble irritants were injected into the pleural cavity and compared for their ability to affect prostanoid concentrations in peritoneal fluids 24 h after treatment. The results presented in Fig. 2 show that the CaPP suspension, dextran solution, saline and phosphate buffered saline induced similar increases in the amount of each prostanoid when compared to untreated controls.

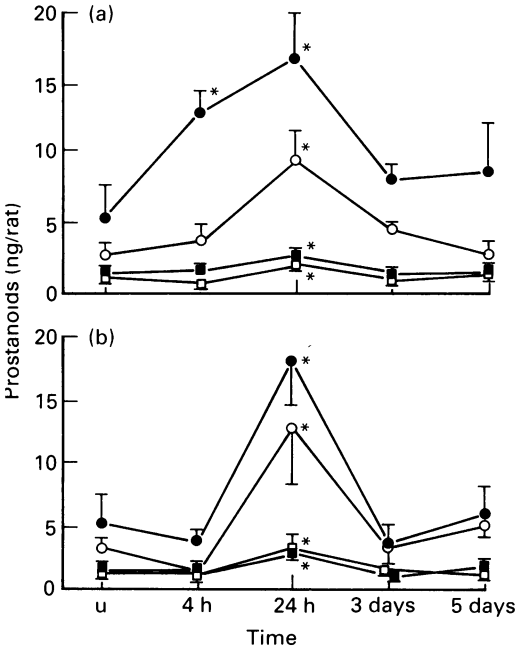

Fig. 1. Prostanoids release *in vivo*. ■, PGE_2; □, $PGF_{2\alpha}$; ●, 6-keto-$PGF_{1\alpha}$; ○, TXB_2 in lavages of the peritoneal cavity of rats, 4 h to 5 days after an intrapleural injection of a, CaPP; b, saline or in lavages from untreated rats (u). Results are expressed as ng/rat (Mean ± s.e.m., $n = 6$–8 rats for each time); $^*P < 0.05$.

In-vitro release of prostanoids by peritoneal macrophages

Peritoneal macrophages from rats having undergone intrapleural injections of various types of irritant were compared for their ability to secrete prostanoids *in vitro* in the absence of any other stimulus.

As shown in Fig. 3a, from day 3 to day 5 after intrapleural injection of dextran, peritoneal macrophages liberated higher amounts

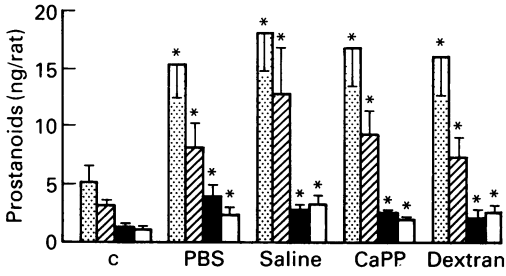

Fig. 2. Prostanoids release *in vivo*. ▨, 6-keto-$PGF_{1\alpha}$; ▨, TBX_2; ■, PGE_2; □, $PGF2_\alpha$ in lavages of the peritoneal cavity of rats, 24 h after an intrapleural injection of different substances or in lavages from untreated controls (c). Results are expressed as ng/rat (Mean ± s.e.m., $n = 6$–8 rats for each time); $^*P < 0.05$

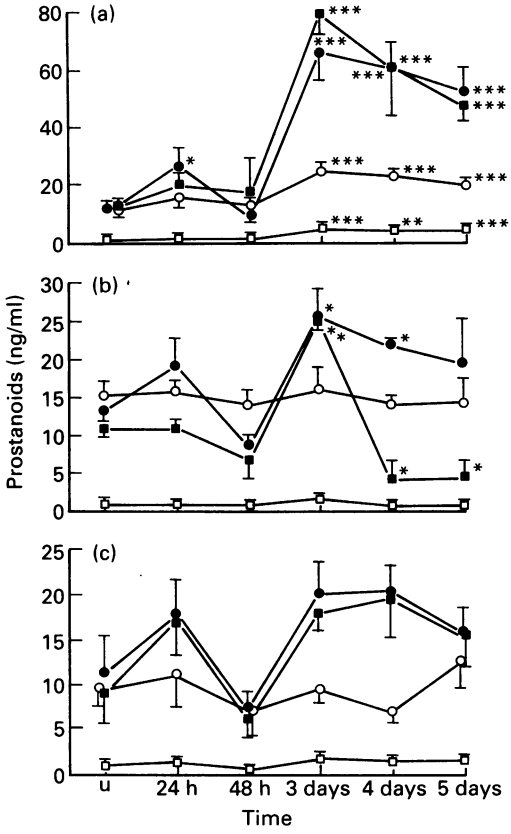

Fig. 3. Prostanoids levels *in vitro*. ■, PGE_2; □, $PGF_{2\alpha}$; ●, 6-keto-$PGF_{1\alpha}$; ○, TBX_2 in supernatants of rat peritoneal macrophages harvested 24 h to 5 days after intrapleural injections of a, dextran; b, CaPP or c, saline or collected from untreated rats (u), and cultured for 24 h. Results are expressed as ng/ml (Mean ± s.e.m., $n=6$ rats for each time) where* $P=<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$ compared to control values (u).

of the four prostanoids than macrophages from untreated controls in 24-h culture supernatants. This production was highest on day 3 and the increase in PGE_2 and 6-keto-$PGF_{1\alpha}$ production was greater than that in TXB_2 and $PGF_{2\alpha}$. In addition, 6-keto-$PGF_{1\alpha}$ release was also significantly augmented 24 h after pleurisy induction.

When CaPP was used as the inflammatory stimulus, slightly different results were obtained (Fig. 3b). Only PGE_2 and 6-keto-$PGF_{1\alpha}$ secretion by cultured peritoneal macrophages was enhanced and again the highest levels were attained on day 3 after pleurisy induction. While 6-keto-$PGF_{1\alpha}$ production remained significantly increased on day 4 and returned to control values on day 5, the levels of PGE_2 on days 4 and 5 were significantly lower than in supernatants of peritoneal macrophages from untreated control rats. No significant variation in $PGF_{2\alpha}$ or TXB_2 levels in macrophage supernatants was observed at any time studied. The amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ liberated by macrophages collected 3 days after pleurisy induction were two to three-fold higher after dextran than after CaPP injection.

Peritoneal macrophages from rats having undergone intrapleural saline injections did not exhibit significant changes in prostanoid release *in vitro* irrespective of the time of cell harvest, as compared to macrophages from control rats (Fig. 3c). A similar absence of effect was observed after intrapleural injection of phosphate buffered saline (results not shown).

Discussion

In the present study, we demonstrate in the rat that during the development of an acute inflammatory reaction in the pleural cavity, cells present at a distant site (peritoneal cavity) display an increased capacity to release prostanoids.

In untreated rats, basal levels of prostanoids produced by peritoneal cells were low and relatively constant from one animal to another. In rats with pleural inflammation, enhanced levels of PGI_2 were observed both in peritoneal lavages (experiments *in vivo*) and in macrophage supernatants after 24-h culture (experiments *in vitro*). TBX_2 levels were mainly increased in peritoneal lavages and PGE_2 in culture supernatants. $PGF_{2\alpha}$ modifications were small.

Increased levels of PGI_2 appeared earlier than those of the three other mediators: as early as 4 h *in vivo* and 24 h *in vitro* after the induction of pleurisy. Levels of the four

prostanoids in the peritoneal cavity reached a maximum *in vivo* 24 h after the induction of pleurisy; *in vitro*, the highest amounts appeared in culture media of peritoneal macrophages harvested 3 days after the injection of the irritant. These differences between the results *in vivo* and *in vitro* could be explained by indirect stimulation and perhaps by variations in the cell population. In effect, the cultured cells were solely macrophages, known to release PGE_2 preferentially. *In vivo* only 66% of the cells present are macrophages, the rest being composed of neutrophils, mesothelial cells, eosinophils, lymphocytes and mast cells (Bachelet *et al.* 1987), each group releasing different mediators preferentially.

In the experiments *in vitro*, similar results were obtained using CaPP or dextran as irritants, although the effects of the latter were stronger. However, CaPP crystals are nondiffusable and cannot therefore directly influence cells in distant parts of the organism. Following the intrapleural injection of PBS or saline, variations in prostanoid levels were not significant compared to untreated rats. *In vivo*, however, prostanoid levels in lavages of the peritoneal cavity were of the same magnitude regardless of the stimulus.

It is known that even slight irritants (saline, PBS) induce modifications in leucocyte reactivity as measured by chemiluminescence after pleural injection (Bird & Giroud 1984). Variations have also been observed (Sultan *et al.* 1978) in the number of leucocytes in the pleural cavity soon after the injection of saline. This is why untreated animals were used as controls.

Other authors have studied the production of eicosanoids by resident macrophages or peritoneal cells elicited by various stimuli. The control levels given by different authors for resident macrophages obtained by lavage and cultured *in vitro* are very similar to ours (Bonney & Humes 1984; Brune & Peskar 1985; Cook *et al.* 1981; Glatt *et al.* 1977; Scott *et al.* 1982). These levels are reported to increase significantly in cultured macrophages harvested at the site of the inflammation. However, prostanoid production in the above studies was triggered *in vitro* by an additional stimulus: zymosan, LPS, PMA, calcium ionophore or components of the complement system (Bockman 1981; Bonney & Humes 1984; Cook *et al.* 1981; Davidson *et al.* 1980; Drapier *et al.* 1983; Feuerstein *et al.* 1981; Gemsa *et al.* 1978; 1982; Hänsch *et al.* 1984; Humes *et al.* 1977, 1982; Kurland & Bockman 1978; Ouwendijk *et al.* 1986). In the present work we show that macrophages remote from the inflammatory site release protanoids *in vivo* and *in vitro* without any additional stimulus.

We had previously observed modifications of peritoneal macrophage reactivity during acute pleurisy, e.g. production of increased amounts of interleukin-1 (Bird *et al.* 1985a; Yao *et al.* 1984), modulation of oxidative metabolism (Bird *et al.* 1985b, 1985c) and of cytostatic activity (Giroud *et al.* 1981).

In this work we report enhanced secretion of prostanoids, *in vivo* with all the irritants studied and *in vitro* with dextran and CaPP. The increased production of prostanoids, particularly of PGE_2, could explain modulations of some immunological functions observed in the same experimental model; responsiveness to mitogenic stimulation, cytotoxic T-cell differentiation and NK-cell activity (Giroud *et al.* 1981; Florentin *et al.*, 1985), functions all known to be negatively regulated by PGE_2 (Brunda *et al.* 1980; Goodwin & Ceuppens 1983).

Thus, an acute non-immunological inflammatory reaction causes modifications in prostanoid release by peritoneal cells at at distance from the site of inflammation for several days after the resolution of the local inflammatory process. Moreover, even a slight stimulus can have important pathophysiological consequences locally and at a distance.

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