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Induction of cyclo-oxygenase by interleukin-1 in rheumatoid synovial cells

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The ability of interleukin-1 (IL-1) to stimulate prostaglandin E_2 (PGE_2) production by human rheumatoid adherent synovial cells was found to be time-dependent and sensitive to protein synthesis inhibitors. Cells incubated with exogenous arachidonic acid (10 μM) showed no increase in PGE_2 production. However, with IL-1 (2.5 U/ml) and exogenous arachidonic acid there was a marked increase, with levels reaching twice that for cells incubated with IL-1 alone. Aspirin pre-treatment studies and the use of [*acetyl*-^{14}C]aspirin showed that IL-1 increased PGE_2 production through the induction of cyclo-oxygenase.

Interleukin-1; Prostaglandin E_2; Cyclo-oxygenase; (Human rheumatoid synovial cell)

1. INTRODUCTION

Interleukin-1 is a polypeptide produced by macrophages which has potent amplifying effects on the inflammatory response [1]. One of its most striking properties is the induction of eicosanoid production, in particular PGE_2 [2], in several cell types, such as RASC. As PGE_2 itself is known to be important in the regulation of inflammatory processes [3] this effect might well be an important one in acute episodes of joint inflammation, particularly in the light of recent findings which have indicated elevated levels of IL-1 in rheumatoid joint synovial fluid [4]. As little is known about how IL-1 achieves this effect, we have attempted to unravel part of the mechanism underlying this action, using cultured human RASC. The key controlling event in prostaglandin formation is usually interpreted as being activation of phospholipase A_2. However, using cultured human RASC, we have shown that IL-2 causes PGE_2 production through the induction of the enzyme cyclo-oxygenase, therefore phospholipase A_2 activation does not appear to be the only limiting factor.

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Abbreviations: IL-1, interleukin-1; RASC, rheumatoid adherent synovial cells; PGE_2, prostaglandin E_2; DMEM, Dulbecco's modified Eagle's medium

2. EXPERIMENTAL

2.1. *Cells*

RASC were prepared as described [5] and grown to confluence in 24-well macrowell plates (Linbro) in DMEM (Gibco) supplemented with 10% heat-inactivated foetal calf serum (Gibco). 4th passage cells were used in all experiments, as low levels of spontaneously produced PGE_2 were detectable.

2.2. *PGE_2 production*

IL-1 (2.5 U/ml, Genzyme Ultrapure) was incubated with the cells (1.2×10^5 per 500 μl well) and supernatants were removed at fixed times. The level of PGE_2 in the supernatants was determined by radioimmunoassay as described by Jose et al. [6]. Cross-reactivities of the antiserum with other prostaglandins were as follows: PGE_1, 26%; $PGF_{2\alpha}$, 1.1%; TxB_2, <0.3%; 6-keto-$PGF_{1\alpha}$, 0.03% and with arachidonic acid, 0.06%. Some of

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the experiments were performed in the presence of exogenous arachidonic acid (10 μM, Sigma) which was added to the cells 1 h before determining PGE_2 levels in the supernatant. The effect of cycloheximide (10 μg/ml, Sigma) and actinomycin D (10 μg/ml, Sigma) on the response was also evaluated. Neither showed signs of cytotoxicity as judged by trypan blue exclusion.

2.3. *The use of aspirin*

Aspirin was used to determine the effect of IL-1 on cyclo-oxygenase. Two series of experiments were carried out. Firstly, cells were pretreated for 30 min with aspirin (100 μM, Sigma) to allow the aspirin to inhibit all the cyclo-oxygenase present. Cells were then washed twice with media, IL-1 (2.5 U/ml) added and levels of PGE_2 determined at fixed times (1 h after adding exogenous arachidonic acid (10 μM)) by radioimmunoassay. As aspirin causes an irreversible inhibition of cyclo-oxygenase, prostaglandin production will only occur via the de novo synthesis of cyclo-oxygenase.

In the second series of experiments, [*acetyl*-^{14}C]aspirin (50 μM, spec. act. 25 μCi/mmol, Amersham) was used. IL-1 (2.5 U/ml) was incubated with cells (1×10^6 per incubation) for fixed time periods. [*acetyl*-^{14}C]Aspirin was then added for 30 min at 37°C. Microsomes were prepared by centrifuging the cells at 100000 × *g* for 45 min. Protein was then precipitated from the microsomal pellet and the amount of radioactivity determined. As the aspirin transfers its radioactive acetyl group specifically to cyclo-oxygenase in this protocol [7], the radioactivity measured can be assumed to be due to the amount of cyclo-oxygenase present.

3. RESULTS AND DISCUSSION

Fig.1 shows that unstimulated cells (1.2×10^5 per 500 μl well) produced low levels of PGE_2 (<3 ng·ml^{-1}). IL-1 (2.5 U·ml^{-1}), however, caused a time-dependent production of PGE_2 which increased from 3 ± 1 ng·ml^{-1} ($n = 4$) to 12 ± 2 ng·ml^{-1} ($n = 4$) at 6–8 h incubation and to 54 ± 3 ng·ml^{-1} ($n = 4$) after 24 h. PGE_2 levels began to rise only after 4 h, which is in agreement with Dayer et al. [8] who showed a similar lag phase using mononuclear cell factor (MCF), a macrophage

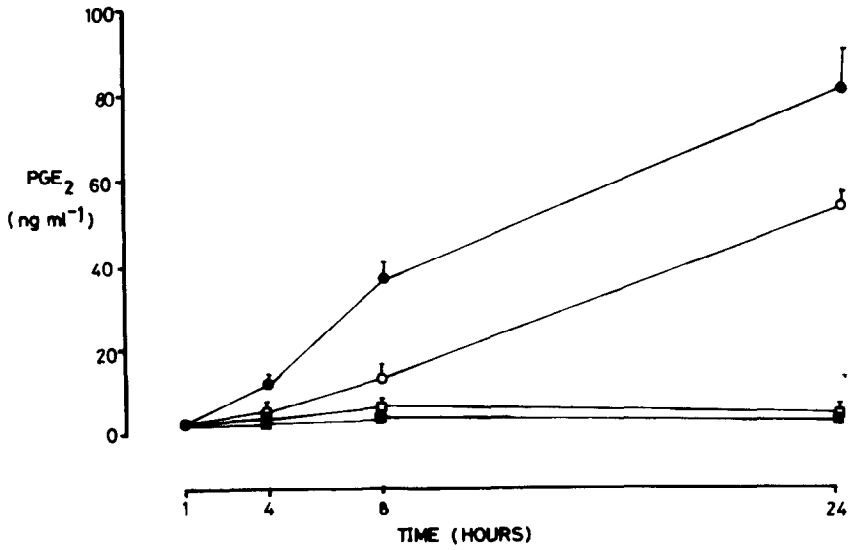

Fig.1. Effect of IL-1 alone and in the presence of arachidonic acid on PGE_2 production by RASC. RASC (6×10^4 cm^{-2}; 2nd–4th subculture) prepared as described [5] were grown to confluence in 24-well macrowell plates (Linbro) in DMEM (Gibco) supplemented with 10% heat-inactivated foetal calf serum (Gibco) and incubated with DMEM alone (■) or IL-1 (2.5 U·ml^{-1}, Genzyme Ultrapure; ○) or with arachidonic acid (10 μM, Sigma; □) or IL-1 with arachidonic acid added 1 h before removal of supernatants (●), for fixed periods of time up to 24 h. Supernatants were then removed and the level of PGE_2 was determined by radioimmunoassay as described by Jose et al. [6].

product which has since been identified as IL-1.

Fig.1 also shows that addition of arachidonic acid (10 μM) to unstimulated cells did not increase PGE_2 production (3 ± 1 ng·ml^{-1}, n = 3) but, when arachidonic acid was added to IL-1-stimulated cells, approximately twice as much PGE_2 was produced (87 ± 10 ng·ml^{-1}, n = 3) as compared with that produced by cells treated with IL-1 alone. These findings indicated that IL-1 was increasing the metabolism of arachidonic acid to PGE_2 and that release of arachidonic acid from membrane phospholipids by phospholipases was not the only controlling event.

The delay of over 4 h in IL-1-induced PGE_2 production was consistent with the view that protein synthesis might be involved in the response. Fig.2a confirms this and shows that the effect was inhibited by the protein synthesis inhibitors, cycloheximide (10 μg·ml^{-1}) and actinomycin D (10 μg·ml^{-1}). Both synthesis inhibitors also blocked IL-1-induced PGE_2 formation by cells to which exogenous arachidonic acid had been added

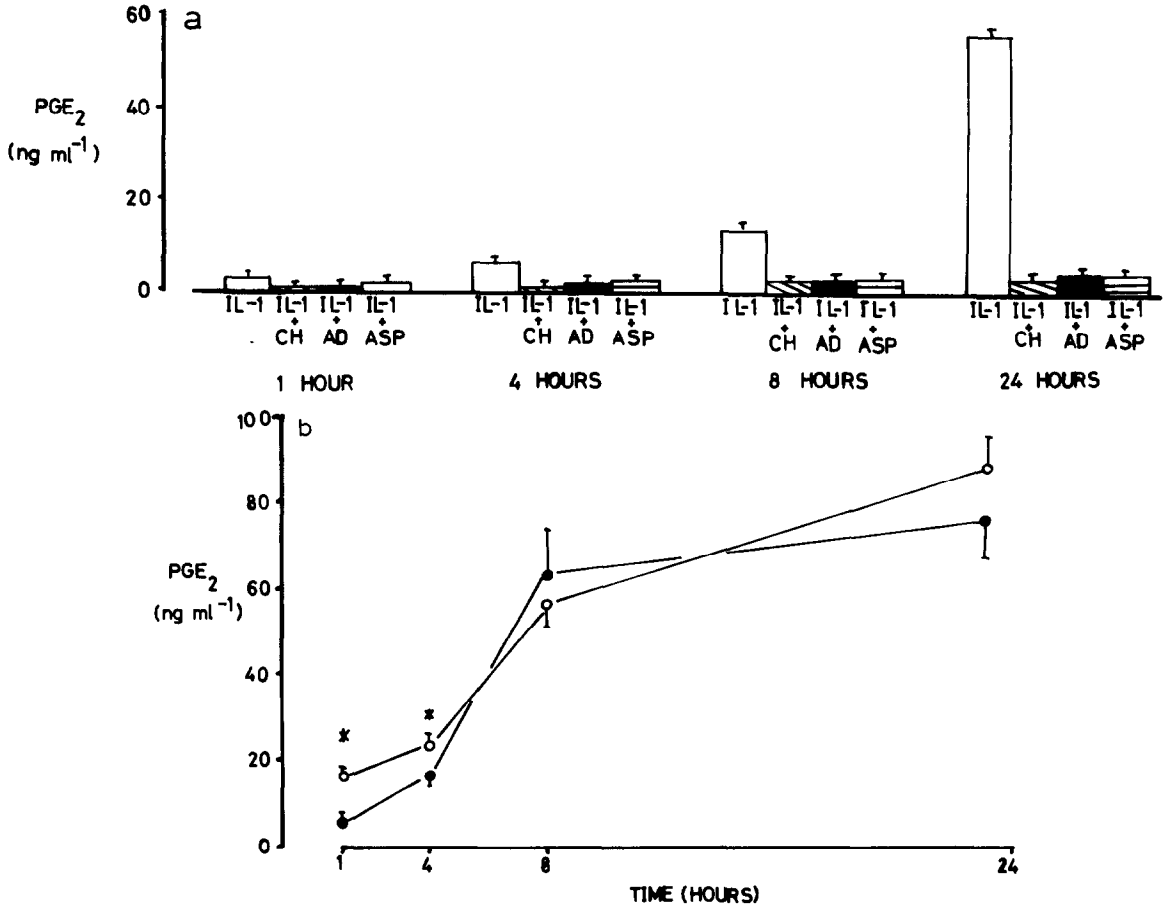

Fig.2. The effect of protein synthesis inhibitors and aspirin on IL-1-induced PGE_2 production by RASC. (a) RASC were incubated with IL-1 (2.5 U·ml^{-1}) and either cycloheximide (CH, 10 μg·ml^{-1}, Sigma), actinomycin D (AD, 10 μg·ml^{-1}, Sigma), aspirin (ASP, 100 μM, Sigma) or DMEM alone. PGE_2 levels were determined by radioimmunoassay in supernatants at the times indicated. Results are the mean ± SE of 3 experiments, each comprising 3 replicates. None of the drugs showed signs of cytotoxicity as judged by trypan blue exclusion. (b) RASC were pretreated for 30 min with either DMEM (○) or aspirin (100 μM) (●) to allow the aspirin to inhibit all cyclo-oxygenase present. Cells were then washed twice with DMEM, IL-1 (2.5 U·ml^{-1}) added and levels of PGE_2 determined at fixed times (1 h after adding exogenous arachidonic acid) by radioimmunoassay. Results are the mean ± SE of 4 experiments, each comprising 3 replicates. A t-test was used to assess the significance of the inhibitory effect of aspirin, * indicates $P < 0.05$.

(not shown). The finding agrees with that of Bernheim and Dinarello [9] who showed the dependence on protein synthesis of IL-1-induced PGE_2 release from human dermal fibroblasts.

It seemed likely from the findings described above that an enzyme subsequent to phospholipase A_2 in the metabolic pathway of arachidonic acid to PGE_2, possibly cyclo-oxygenase, was being induced by IL-1. Two experiments were designed with aspirin, as it is known that aspirin irreversibly inhibits cyclo-oxygenase by acetylating the enzyme.

Fig.2a shows the inhibitory effect of aspirin (100 μM) on the IL-1 response, reducing PGE_2 production by the cells to 5 ± 1 ng·ml^{-1} after 24 h incubation. However, when the cells were pre-incubated with aspirin for 30 min followed by washing to remove free aspirin which had not reacted with cyclo-oxygenase, a different result was obtained (fig.2b). Initially, low amounts of PGE_2 were produced from exogenous arachidonic acid but, after 8 h incubation, PGE_2 production was restored to 57 ± 5 ng·ml^{-1} ($n = 4$) and to 77 ± 7 ng·ml^{-1} ($n = 4$) after 24 h. These levels were similar to those from cells not pre-treated with aspirin. This finding can only be explained by assuming that IL-1 induced de novo synthesis of cyclo-oxygenase. Residual cyclo-oxygenase would be irreversibly acetylated by the aspirin and free aspirin in the culture medium would acetylate most of the newly-formed cyclo-oxygenase. However, when the free aspirin was removed by washing, the newly synthesized cyclo-oxygenase would be available to metabolise arachidonic acid.

The second experiment using aspirin confirmed this finding. It has been shown that incubation with a low concentration of aspirin (50 μM) for a short time (30 min) will result in specific and irreversible acetylation of cyclo-oxygenase [7]. Using [*acetyl*-^{14}C]aspirin, the labelled acetyl group is transferred to cyclo-oxygenase with a 1:1 stoichiometry and thus radioactivity in microsomal protein precipitates gives a measure of the amount of cyclo-oxygenase present. As can be seen in fig.3, radioactivity was increased in microsomal protein precipitates from cells incubated with IL-1. Furthermore, the increase occurred in a time-dependent manner similar to that for PGE_2 production.

The mechanism by which IL-1 stimulates cells

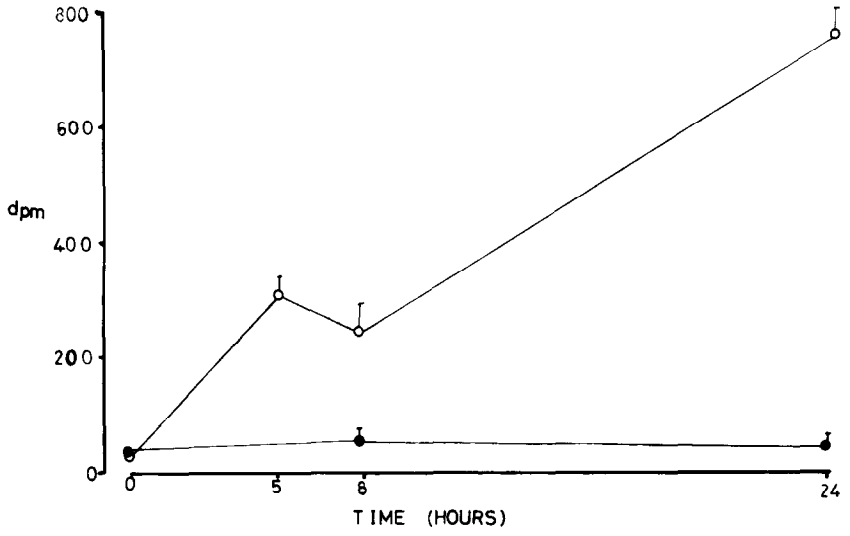

Fig.3. Effect of IL-1 (2.5 U·ml^{-1}) on the amount of cyclo-oxygenase in RASC over 24 h. IL-1 (2.5 U·ml^{-1}; ○) or DMEM alone (●) were incubated with RASC (1×10^6 per incubation) for fixed time periods. Cyclo-oxygenase levels were then determined by incubating the cells with [*acetyl*-^{14}C]aspirin (50 μM, spec. act. 25 μCi·mmol^{-1}, Amersham) for 30 min at 37°C. Microsomes were then prepared and the radioactivity in precipitated protein determined. As the aspirin will transfer its radioactive acetyl group specifically to cyclo-oxygenase in the protocol described [7], radioactivity measured (dpm) can be assumed to be due to the amount of cyclo-oxygenase present. Results are mean ± SE from triplicate determinations.

has not yet been fully elucidated. It appears not to act like some hormones in causing activation of adenylate cyclase or phosphatidylinositol turnover [10]. Recent reports on how IL-1 induces PGE_2 production by chondrocytes have indicated phospholipase A_2 activation [11]. This has also been shown in human synovial cells [12]. Our results suggest that IL-1-induced cyclo-oxygenase production in human RASC may be the more important controlling event. This is similar to the mechanism proposed for other proteins which stimulate eicosanoid production, such as platelet-derived growth factor in mouse 3T3 fibroblasts [13].

These findings not only illustrate the importance of cyclo-oxygenase induction in controlling eicosanoid production, but point to a site of action of IL-1 previously unsuspected, and perhaps suggest enzyme induction as a possible mechanism underlying other actions of IL-1.

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