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The effect of adrenalectomy on interleukin-1 release *in vitro* and *in vivo*

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1 Peritoneal macrophages (Mø) collected from adrenalectomized (ADX) rats released more interleukin-1 (IL-1) activity and prostaglandin E_2 (PGE_2) than macrophages from sham-operated (SHO) rats.

2 The increase in IL-1 activity in the supernatants was confirmed by the increase of the cell-associated 33 kD IL-1α precursor in ADX macrophages stimulated by lipopolysaccharide (LPS).

3 After the injection of Complete Freund's Adjuvant (CFA) to induce adjuvant arthritis, 60% of the ADX rats died, while no deaths occurred in the SHO group.

4 The *in vivo* administration of dexamethasone inhibited both IL-1 and PGE_2 release by macrophages as well as protecting ADX animals from CFA-induced death. Indomethacin and BW 755C partially protected the animals from this lethal effect.

5 These results suggest that adrenalectomy induces an increased release of IL-1 both *in vitro* and *in vivo*, and are consistent with a feedback mechanism between IL-1 and glucocorticoid hormones.

Introduction

The acute inflammatory response is an important part of the body's defence mechanisms against injury or infection. This response has as its main objectives the neutralization and removal of pathogens, the rebuilding of injured tissues and ultimately the restoration of normal structure and function. Sometimes the activation of defence reactions lasts well after the removal of the injurious agents triggering the transition from acute to chronic inflammation. It has been proposed that the rise in adrenocortical steroid hormones occurring during inflammation might turn off the activated defence reactions in order to restore homeostasis (Munck *et al.*, 1984). Accordingly, we have shown previously that the extent of the acute inflammatory response in adrenalectomized rats is much greater than in sham-operated animals (Flower *et al.*, 1986). This phenomenon was sustained by the activation of phospholipase A_2 (PLA_2) with consequent formation of pro-inflammatory eicosanoids. The administration of dexamethasone decreased the severity of the inflammatory process probably via the induction of anti-phospholipase proteins (lipocortins) (Parente & Flower, 1985; Flower *et al.*, 1986).

In recent years the important role of interleukin-1 (IL-1) in inflammation has been well documented (Dinarello, 1988; Cybulsky *et al.*, 1988). It has been shown that IL-1 is able to stimulate prostaglandin formation by both increasing cellular cyclo-oxygenase biosynthesis (Raz *et al.*, 1988) and activating PLA_2 (Burch *et al.*, 1988; Solito & Parente, 1989). On the other hand, IL-1 can directly stimulate the adrenal cortex (Roh *et al.*, 1987) and its synthesis and release is inhibited by glucocorticoids (Snyder & Unanue, 1982; Knudsen *et al.*, 1987; Lew *et al.*, 1988). In order to investigate the relationship between IL-1 and endogenous steroid hormones we have studied the effect of adrenalectomy on IL-1 release and we report that macrophages from ADX rats (hereafter referred to as ADX macrophages) release significantly more IL-1 than macrophages from SHO animals (hereafter referred to as SHO macrophages). This enhanced release may occur also *in vivo* following the challenge with Complete Freund's Adjuvant (CFA).

Methods

Animals

Adrenalectomized and sham-operated outbred Wistar rats (200 ± 10 g body wt) were obtained from

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Charles River, Calco, Italy. ADX rats were given 0.9% NaCl solution to drink, while SHO rats were given tap water. Animals were fed on standard chow pellets *ad libitum* and used for experiments two weeks after surgery.

Cell culture

Resident peritoneal cells were collected by lavage with phosphate-buffered saline (PBS) containing heparin 5 units ml^{-1}. Cells from 5–8 animals were pooled and plated at 2×10^6 cells per well in multiwell plates 24 × 10 mm (Costar, Cambridge, MA, USA) and incubated in 0.5 ml of RPMI 1640 (Gibco, Paisley, U.K.) containing L-glutamine 2 mM, HEPES buffer 25 mM, and gentamicin sulphate 50 $\mu g\,ml^{-1}$ (Sigma Chemical Co., St. Louis, MO, U.S.A.) and supplemented with 20% Foetal Calf Serum (FCS, Seromed, Berlin, FRG). After 2 h incubation at 37°C in 5% CO_2 atmosphere, nonadherent cells were removed by washing thoroughly with serum-free RPMI and the adherent population was further incubated in RPMI 1640 + 5% FCS, either with or without 10 $\mu g\,ml^{-1}$ lipopolysaccharide (LPS) from *E. coli* (055:B5, Difco, Detroit, MI, U.S.A.). Adherent cells were shown to be >98% macrophages by positive esterase staining. After 24 h incubation, supernatant aliquots were collected for PGE_2 assay, the remaining supernatants were dialyzed against PBS (1:200 v/v) twice and sterile filtered.

Prostaglandin E_2 assay

Concentrations of PGE_2 in the supernatants were determined by specific radioimmunoassay with minimal (3.7%) cross-reactivity with PGE_1 (N.E.N., Dreieich, FRG).

Interleukin-1 assay

IL-1 activity in dialyzed supernatants was assessed by the murine thymocyte co-stimulation assay according to Massone *et al.* (1988). Briefly, 6×10^5 thymocytes from C3H/HeJ mice were incubated in multiwell plates (Costar) in 0.2 ml of RPMI 1640 5% FCS with 1.5 $\mu g\ ml^{-1}$ phytohaemoagglutinin (PHA, The Wellcome Foundation, Beckenham, U.K.) and serial twofold dilutions of the supernatants. After 72 h cells were pulsed for further 15 h with 1 μCi per well [^{3}H]-thymidine (185 GBq $mmol^{-1}$, Amersham International, U.K.).

Data are presented as units ml^{-1} calculated for sample dilutions giving 50% of the maximal thymidine incorporation compared to standard curves obtained with endotoxin-free human recombinant IL-1β (specific activity 1.10^7 units mg^{-1}, Sclavo, Siena, Italy).

Immunoprecipitation

ADX and SHO peritoneal macrophages (1×10^7 cells in 2 ml) were incubated for 20 h in RPMI 1640 + 10% FCS with or without LPS (10 $\mu g\,ml^{-1}$) in the presence of 1 $\mu g\,ml^{-1}$ cold methionine (Sigma Chemical Co.) and 50 $\mu Ci\,ml^{-1}$ [^{35}S]-methionine (N.E.N.). At the end of the incubation the cells were washed twice with PBS and lysed for 10 min at 25°C in 0.5 ml PBS containing 0.5% Nonidet P-40 (LKB, Bromma, Sweden and 1 mM phenylmethylsulphonylfluoride (PMSF). The cell lysates were spun at 10,000 *g* for 10 min and cleared with 5 μl of normal rabbit serum followed by 100 μl of protein A coupled to Sepharose 4B (Pharmacia, Uppsala, Sweden). To the cleared lysates were added 1:100 dilutions of either rabbit polyclonal anti-human recombinant IL-1β (Massone *et al.*, 1988) or rabbit polyclonal anti-human recombinant IL-1α (Genzyme Biochemicals, Maidstone, U.K.). After incubation for 18 h at 4°C, 100 μl of protein A Sepharose 4B was added, and the immunoprecipitates were washed 3 times in Tris-HCl 10 mM pH 7.5 containing NaCl 150 mM and PMSF 4 $\mu g\,ml^{-1}$, followed by three washes with the same buffer containing NaCl 500 mM and finally by 3 washes with the same buffer without NaCl. The immunoprecipitated material was subjected to electrophoresis on 15% SDS polyacrylamide gels (LKB) according to Laemmli (1970). The gels were fixed with 30% methanol, enhanced with Enlightning (N.E.N.), vacuum dried and exposed to photographic film (Kodak X-Omat) at −40°C for 48 h.

In vivo *experiments*

In order to induce adjuvant arthritis ADX and SHO rats were injected s.c. with Complete Freund's Adjuvant (0.2 ml, 10 mg ml^{-1}, *M. tuberculosis*, Difco) into the right hind paw.

Drugs

Dexamethasone 21-acetate and indomethacin were obtained from Sigma Chemical Co. BW 775C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2 pyrazoline) was kindly supplied by The Wellcome Foundation. On the basis of preliminary results, in the *ex vivo* experiments dexamethasone (0.1 mg kg^{-1}, s.c.) was administered for 9 days and macrophages collected 24 h after the last administration (see Figure 1).

Statistics

Statistical analysis was performed using Student's *t* test for unpaired samples with $P < 0.05$ regarded as significant.

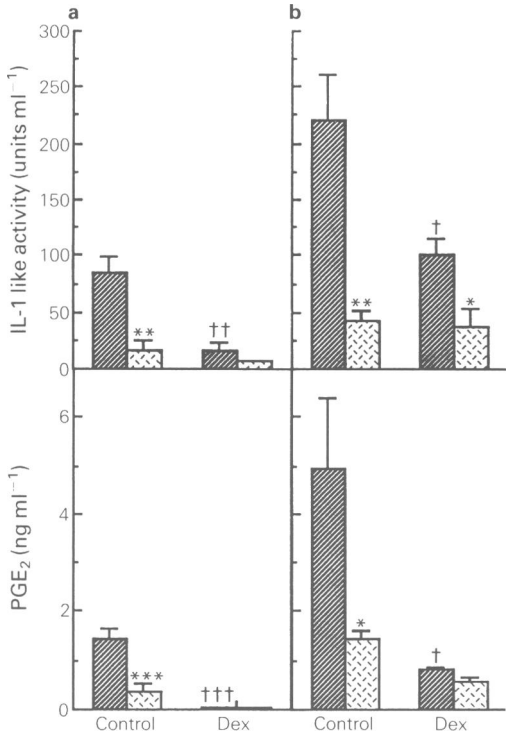

Figure 1 Interleukin-1 (IL-1)-like activity (upper panel) and prostaglandin E_2 (PGE_2) concentration (lower panel) in supernatants of peritoneal macrophages collected from adrenalectomized (hatched columns) and sham-operated (stippled columns) rats. Animals were treated either with vehicle 1 ml kg^{-1} s.c. (Control) or with dexamethasone 0.1 mg kg^{-1} s.c. (Dex) for 9 days and macrophages were collected 24 h after last administration. (a) Unstimulated macrophages; (b) Lipopolysaccharide-stimulated macrophages. Columns represent mean (with s.e.mean shown by vertical bars) of one experiment performed in triplicate. Four other experiments gave similar results. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$ vs corresponding ADX group. † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.005$ vs control ADX group.

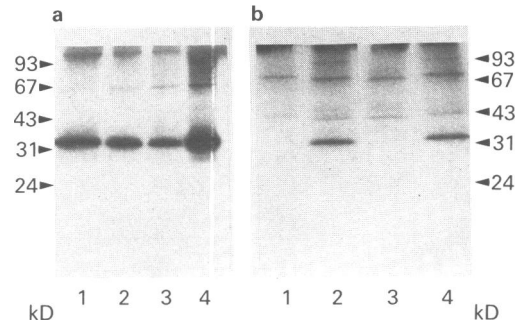

Figure 2 Immunoprecipitation pattern of macrophage lysates from sham-operated (lanes 1 and 2) and adrenalectomized rats (lanes 3 and 4). Macrophages were incubated for 20 h either in absence (lanes 1 and 3) or in presence of 10 μg ml^{-1} lipopolysaccharide (lanes 2 and 4). Antibodies used were rabbit polyclonal anti-hr interleukin-1α (IL-1α, a) and rabbit polyclonal anti-hr IL-1β (b).

Results

Interleukin-1 and prostaglandin E_2 release by macrophages

Control peritoneal macrophages from ADX rats secreted into the culture medium significantly more IL-1 activity than those from SHO animals (Figure 1a, upper panel). LPS stimulation increased the absolute amount of IL-1 released by either type of macrophage, leaving the proportional difference between ADX and SHO macrophages virtually unchanged (Figure 1b, upper panel). The *in vivo* administration of dexamethasone significantly reduced IL-1 release by ADX and SHO macrophages whether stimulated or not by LPS. Moreover, control peritoneal macrophages from ADX rats released significantly more PGE_2 than those from SHO animals (Figure 1a, lower panel). As with IL-1, PGE_2 biosynthesis was greatly enhanced by LPS stimulation (Figure 1b, lower panel) and was reduced by *in vivo* administration of dexamethasone.

Interleukin-1 immunoprecipitation

In order to investigate the involvement of the two molecular species of IL-1 in the biological activity observed in the macrophage supernatants, we carried out immunoprecipitation experiments using specific polyclonal antibodies raised against human recombinant IL-1α and IL-1β. The results show that the anti-IL-1α antibody detected the presence of cell-associated 33 kD IL-1α in macrophages whether stimulated or not by LPS (Figure 2a). The largest amount of pro-IL-1α was observed in ADX macrophages stimulated by LPS (Figure 2a, lane 4). On the other hand, the anti-IL-1β antibody was able to recognize the cell-associated pro-IL-1β only upon stimulation with LPS (Figure 2b). No evident difference in the band corresponding to the 33 kD pro-IL-1β could be observed between SHO and ADX macrophages. It is also clear that rat macrophages express more pro-IL-1α than pro-IL-1β. Finally, we were unable to observe immunoprecipitation of soluble IL-1 in the macrophage supernatant (data not shown).

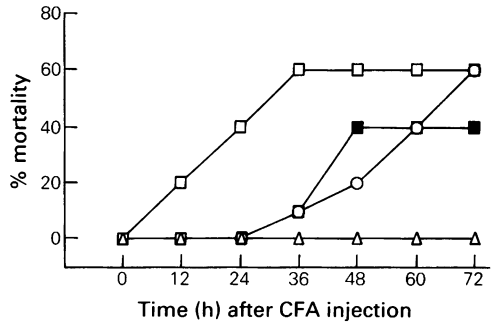

Figure 3 Cumulative mortality in adrenalectomized rats ($n = 10$) injected with Complete Freund's Adjuvant (CFA) at time 0. Drugs were administered at 0, 4 and 8 h. Control group (□) received vehicle 1 ml kg^{-1} orally; (○) indomethacin 3 mg kg^{-1} orally; (■) BW755C 20 mg kg^{-1} orally; (△) dexamethasone 1 mg kg^{-1} s.c.

In vivo *experiments*

We have shown elsewhere that macrophages collected from rats with adjuvant arthritis release more IL-1 than those from control animals (Becherucci *et al.*, 1989). In order to investigate the effect of adrenalectomy on IL-1 release in arthritic animals CFA was injected in ADX and SHO rats. Thirty-six hours after the injection, 60% of ADX rats died (Figure 3). No rats died either in the SHO group or in ADX animals injected with Incomplete Freund's Adjuvant (not shown). The lethal effect of CFA injection was completely reversed by dexamethasone given three times on the first day. Indomethacin and BW755C, given according to the same dosing schedule as the steroid, protected only up to 24 h after CFA injection (Figure 3).

Discussion

We have previously proposed that the enhancement of the inflammatory response in ADX animals is at least partially due to the activation of PLA_2 not counteracted by an increase in circulating corticosteroid hormones. This hypothesis was based on the observation that inflammatory exudates from ADX rats contain significantly higher levels of eicosanoids and macrophages collected from ADX rats release more lipid mediators than SHO animals (Parente & Flower, 1985; Flower *et al.*, 1986). Our present results show that macrophages collected from ADX animals release more IL-1 and PGE_2 than control cells from SHO animals. As IL-1 stimulates PLA_2 (Burch *et al.*, 1988; Solito & Parente, 1989) it is conceivable that the enzyme activation is secondary to the increase of IL-1 synthesis. In order to investigate the IL-1 release during an inflammatory process we decided to induce adjuvant arthritis in ADX and SHO rats, since we have shown elsewhere that macrophages from arthritic animals release more IL-1 than control cells (Becherucci *et al.*, 1989). Unexpectedly, 36 h after CFA injection 60% of the ADX rats died, while no death occurred in the SHO animals. This is consistent with the recent observation that IL-1 and tumour necrosis factor (TNF) are lethal for adrenalectomized mice but not for sham-operated ones (Bertini *et al.*, 1988). It is tempting to hypothesize that the CFA injection prompted the release of IL-1 by macrophages and other IL-1-forming cells *in vivo* causing the death of ADX rats. The involvement of TNF in this phenomenon is currently under investigation.

In summary our results may suggest that adrenalectomy increases the release of IL-1 both *in vitro* and *in vivo*. Several investigators have shown that glucocorticoids suppress IL-1 synthesis by blocking the transcription of IL-1 mRNA (Knudsen *et al.*, 1987; Lew *et al.*, 1988; Lee *et al.*, 1988). Therefore, the synthesis of IL-1 in ADX animals may be deregulated because of the absence of endogenous corticosteroid hormones. The *in vivo* administration of dexamethasone was indeed able to inhibit both IL-1 and PGE_2 release by macrophages, as well as exhibiting a long-lasting protective effect from CFA-induced death in ADX rats. Indomethacin, a cyclo-oxygenase inhibitor, and BW755C, a dual cyclo-oxygenase/lipoxygenase inhibitor, given three times on the first day, only protected up to 24 h following CFA injection (see Figure 3). We have subsequently observed that three daily administrations of the non-steroid drugs for three days completely protected the ADX rats from death (data not shown). This suggests that CFA injection caused a sustained biosynthesis of toxic eicosanoids. Therefore, we would like to propose that in ADX rats injected with CFA, a continuous release of IL-1 occurs with subsequent activation of PLA_2, which may cause directly a circulatory collapse (Vadas *et al.*, 1988) as well as induce the formation of toxic metabolites like thromboxanes and platelet activating factor (PAF). It has been shown that a shock-like state caused by IL-1 in rabbits was abolished by cyclo-oxygenase inhibitors (Okusawa *et al.*, 1988). Moreover, IL-1 stimulated PAF formation (Bussolino *et al.*, 1986) and a PAF antagonist blocked IL-1-induced inflammatory effects (Rubin & Rosenbaum, 1988). In this light the long-lasting protective effect of dexamethasone can be ascribed to the combined inhibition of IL-1 synthesis and induction of anti-PLA_2 proteins. Indeed, we have recently shown that in human fibroblasts, hydrocortisone blocks IL-1-induced PLA_2 activation through the induction of lipocortins (Solito & Parente, 1989). The enhanced synthesis

and release of IL-1 following adrenalectomy and the inhibitory effect of glucocorticoids are consistent with the hypothesis that endogenous steroid hormones control homeostasis by lowering the intensity of the body's defence reactions (Munck *et al.*, 1984).

It has been shown that IL-1 stimulates the secretion of both corticotropin-releasing factor (Salposki *et al.*, 1987; Berkenbosch *et al.*, 1987) and adrenocorticotrophic hormone (ACTH, Besedowsky *et al.*, 1986). Moreover, IL-1 increases rat serum corticosterone levels by direct stimulation of the adrenal cortex (Roh *et al.*, 1987). In view of these observations, an immunoregulatory feedback mechanism between IL-1 and glucocorticoids has been proposed by Besedowski *et al.* (1986). Our results provide a physiological basis for this feedback mechanism, showing, for the first time, the modulation of IL-1 release by endogenous steroid hormones.

Immunoprecipitation of IL-1 synthesized in peritoneal macrophages shows that IL-1α is constitutively produced and in larger amounts than IL-1β, which is synthesized only after stimulation by LPS. Interestingly, LPS-stimulated macrophages from ADX rats appeared to have the highest quantity of the IL-1α precursor. Since it has been shown that only the pro-IL-1α is biologically active (Mosley *et al.*, 1987) it is possible that the enhanced IL-1-like activity released by ADX macrophages is due to this form of the interleukin. It has been also reported that rat macrophages release more IL-1α than IL-1β (Kampschmidt & Franks, 1985). No extracellular forms of IL-1 were observed in the immunoprecipitation gels. It is likely that the bioassay is able to detect IL-1 amounts not revealed by immunoprecipitation because of different sensitivity, as previously reported (Hazuda *et al.*, 1988). However, the involvement of other macrophage products in the observed biological activity cannot, at the moment, be excluded.

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References

BECHERUCCI, C., DONATI, D., PERRETTI, M. & PARENTE, L. (1989). The release of interleukin-1-like activity by macrophages in two models of immunological inflammation in the rat. *Agents Actions*, (in press).

BERKENBOSCH, F., VAN OERS, J., DEL REY, A., TILDERS, F. & BESEDOVSKI, H. (1987). Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science*, **238**, 524–526.

BERTINI, R., BIANCHI, M. & GHEZZI, P. (1988). Adrenalectomy sensitizes mice to the lethal effects of interleukin-1 and tumor necrosis factor. *J. Exp. Med.*, **167**, 1708–1712.

BESEDOWSKY, H., DEL REY, A., SORKIN, E. & DINARELLO, C.A. (1986). Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science*, **233**, 652–654.

BURCH, R.M., CONNOR, J.R. & AXELROD, J. (1988). Interleukin-1 amplifies receptor-mediated activation of phospholipase A_2 in 3T3 fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 6306–6309.

BUSSOLINO, F., BREVIARIO, F., TETTA, C., AGLIETTA, M., MANTOVANI, A. & DEJANA, E. (1986). Interleukin-1 stimulates platelet-activating factor production in cultured human endothelial cells. *J. Clin. Invest.*, **77**, 2027–2033.

CYBULSKY, M.I., CHAN, M.K.W. & MOVAT, H.Z. (1988). Acute inflammation and microthrombosis induced by endotoxin, interleukin-1, tumour necrosis factor and their implication in gram-negative infection. *Lab. Invest.*, **58**, 365–378.

DINARELLO, C.A. (1988). Biology of interleukin 1. *FASEB J.*, **2**, 108–115.

FLOWER, R.J., PARENTE, L., PERSICO, P. & SALMON, J.A. (1986). A comparison of the acute inflammatory response in adrenalectomized and sham-operated rats. *Br. J. Pharmacol.*, **87**, 57–62.

HAZUDA, D.J., LEE, J.C. & YOUNG, P.R. (1988). The kinetics of interleukin-1 secretion from activated monocytes. Differences between interleukin-1α and interleukin-1β. *J. Biol. Chem.*, **263**, 8473–8479.

KAMPSCHMIDT, R.F. & FRANKS, E. (1985). The physical and biological properties of rat interleukin-1. *J. Leuk. Biol.*, **37**, 715.

KNUDSEN, P.J., DINARELLO, C.A. & STROM, T.B. (1987). Glucocorticoids inhibit transcriptional and post-transcriptional expression of interleukin-1 in U937 cells. *J. Immunol.*, **139**, 4129–4134.

LAEMMLI, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

LEE, S.W., TSOU, A.-P., CHAN, H., THOMAS, J., PETRIE, K., EUGUI, E.M. & ALLISON, A.C. (1988). Glucocorticoids selectively inhibit the transcription of the interleukin-1β gene and decrease the stability of interleukin-1β mRNA. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 1204–1208.

LEW, W., OPPENHEIM, J.J., MATSUSHIMA, K. (1988). Analysis of the suppression of IL-1α and IL-1β production in human peripheral blood mononuclear adherent cells by a glucocorticoid hormone. *J. Immunol.*, **140**, 1895–1902.

MASSONE, A., BALDARI, C., CENSINI, S., BARTALINI, M., NUCCI, D., BORASCHI, D. & TELFORD, J.L. (1988). Mapping of biologically relevant sites on human IL-1β using monoclonal antibodies. *J. Immunol.*, **140**, 3812–3816.

MOSLEY, B., URDAL, D.L., PRICKET, K.S., LARSEN, A., COSMAN, D., CONLON, P.J., GILLIS, S. & DOWER, S.K. (1987). The interleukin-1 receptor binds the human interleukin-1α precursor but not the interleukin-1β precursor. *J. Biol. Chem.*, **262**, 2941–2944.

MUNCK, A., GUYRE, P.M. & HOLBROOK, N.J. (1984). Physiological functions of glucocorticoids in stress and

their relation to pharmacological actions. *Endocrine Rev.*, **5**, 25–44.

OKUSAWA, S., GELFAND, J.A., IKEJIMA, T., CONNOLLY, R.J. & DINARELLO, C.A. (1988). Interleukin-1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. *J. Clin. Invest.*, **81**, 1162–1172.

PARENTE, L., FLOWER, R.J. (1985). The generation of lyso-PAF in experimental inflammation. In *Inflammatory Mediators*. ed. Higgs, G.A. & Williams, T.J. pp. 65–71. Basingstoke: Macmillan Press.

RAZ, A., WYCHE, A., SIEGEL, N., NEEDLEMAN, P. (1988). Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. *J. Biol. Chem.*, **263**, 3022–3028.

ROH, M.S., DRAZENOVICH, K.A., BARBOSE, J.J., DINARELLO, C.A. & COBB, C.F. (1987). Direct stimulation of the adrenal cortex by interleukin-1. *Surgery*, **102**, 140–146.

RUBIN, R.M. & ROSENBAUM, J.T. (1988). A platelet-activating factor antagonist inhibits interleukin-1-induced inflammation. *Biochem. Biophys. Res. Commun.*, **154**, 429–436.

SALPOSKI, R., RIVIER, C., YAMAMOTO, G., PLOTSKI, P. & VALE, W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science*, **238**, 522–524.

SNYDER, D.S. & UNANUE, E.R. (1982). Corticosteroids inhibit murine macrophage Ia expression and interleukin-1 production. *J. Immunol.*, **129**, 1803–1805.

SOLITO, E. & PARENTE, L. (1989). Modulation of phospholipase A_2 activity in human fibroblasts. *Br. J. Pharmacol.*, **96**, 656–660.

VADAS, P., PRUZANSKI, W. & STEFANSKI, E. (1988). Extracellular phospholipase A_2: causative agent in circulatory collapse of septic shock. *Agents Actions*, **24**, 320–325.

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