Role of protein tyrosine phosphorylation on COX-2 induction in endotoxin-activated endothelial cells and macrophages: a comparison with nitric oxide synthase

P. Akarasereenont, J. A. Mitchell, I. Appleton, C. Thiemermann and J. R. Vane
The William Harvey Research Institute, St Bartholomews' Hospital Medical College, Charterhouse Square, London EC1M 6BQ, UK

Cyclooxygenase (COX) and nitric oxide synthase (NOS) are two enzymes which have distinct cytokine-inducible isoforms (COX-2 and iNOS, respectively). Many cytokine receptors have an intracellular tyrosine kinase domain. Here, we have used the tyrosine kinase inhibitors, erbstatin and genistein, to investigate the potential role of tyrosine kinase activation in the induction of COX-2 and iNOS caused by endotoxin (lipopolysaccharide; LPS at 1 μg/ml for 24 h) in bovine aortic endothelial cells (BAEC) and J774.2 macrophages. The predominant COX metabolites, 6-oxo-prostaglandin (PG) F_2α (for BAEC) and PGE_2 (for J774.2 macrophages) were measured by radioimmunoassay under the following experimental conditions: (i) accumulation of COX metabolites from endogenous arachidonic acid was measured at 24 h after addition of LPS (1 μg/ml); (ii) in experiments designed to measure 'COX activity', COX metabolites generated by LPS-activated BAEC or J774.2 macrophages were assayed after incubation with exogenous arachidonic acid (30 μM for 15 min). Western blot analysis with a specific antibody to COX-2 was used to determine the expression of COX-2 protein caused by LPS in cell extracts. Accumulation of nitrite (measured by the Griess reaction) was used to measure NOS activity. Erbstatin or genistein also caused a dose-dependent inhibition of nitrite accumulation in J774.2 macrophages activated with LPS (1 μg/ml for 24 h). In contrast to J774.2 macrophages, BAEC stimulated with LPS did not produce detectable amounts (<1 μM) of nitrite. These results show that tyrosine phosphorylation is part of the signal transduction mechanism that mediates (i) the induction of COX-2 and iNOS elicited by LPS in J774.2 macrophages, and (ii) the induction of COX-2 by LPS in BAEC.

Inhibition of the local Shwartzman reaction in rabbits by CSVTCG, a thrombospondin-derived hexapeptide

C. E. Burrowes, S. Peers, L. Tran, and B. Beaubien
Allelix Biopharmaceuticals Inc., 6850 Goremway Drive, Mississauga, Ontario, Canada L4V 1P1

Thrombospondin (TSP) is a multidomain protein involved in cell adhesion processes through its associations with CD36 (GPIV, GPIIIb), αβ3, αβ1, and perhaps other binding proteins. The peptide form of CSVTCG blocks TSP binding to CD36, and is reported to reduce tumour cell adhesion and platelet aggregation. The peptide CSVTCG blocks TSP-induced cell–cell associations. We were interested in determining whether this peptide might reduce microvascular pathophysiology, and thus tested it in a model of dermal thrombo-haemorrhage, the local Shwartzman reaction (LSR), in rabbits. The LSR was produced by intradermal injection of endotoxin (preparative dose) followed by intravenous administration of endotoxin (provocative dose) 18-24 h later. Haemorrhagic lesions that developed in the prepared skin sites were quantified by [111]In-labelled erythrocytes. CSVTCG, at a dose of 1.0 mg/kg, given i.v. 15 min before the provocative dose induced a dose-dependent inhibition of haemorrhage (max. 80%, p < 0.05). The same dose of CSVTCG given i.v. 15 min before the preparatory dose was less effective and, when given i.d., had no effect on haemorrhage. These
**Telemetric study of monooiodocetate (MIA) induced osteoarthritis in rats**

D. Chevrier, P. Gegout, C. Guingamp, P. Gillet, B. Terlain and P. Netter

Department of Pharmacology, URA CNRS 1288, BP184, 54505 Nancy, France

Patients’ handicap is the main point during osteoarthritis (OA). Telemetry, in automatically recording mobility of laboratory animals, allows measurement of incapacity during experimentally induced OA, especially in rats. As surgical models are inadapted to telemetry, and after preliminary studies of the impact of various chemical agents on rats’ mobility, spontaneous nocturnal activity was recorded in Wistar rats after i.a. injection of MIA in both knees.

As a result, nocturnal mobility was significantly reduced in the MIA group (3 mg) when compared with controls (saline i.a.). Typically, MIA-induced incapacity advanced in three phases: day 1–3 marked and acute fall; day 4–14: recovery; then from day 15, progressive incapacity. This secondary handicap appears very similar to that observed in human disease; late and slowly progressive oncoming, moderate amplitude, aggravation without related inflammatory phenomena (lack of fever and histological signs). Morphological aspect is in favour of an OA process, showing cartilage erosions and intense osteophytic proliferation.

35S incorporation was assessed ex vivo in a centropatellar punch and in the remaining patella. Loss of centropatellar 35S incorporation seems correlated with the loss of mobility (similar evolution in three phases). Contrasting with this decrease of anabolism in the central punch, the surrounding cartilage exerted an increased anabolism (osteophytes formation). Early, but not late administration, of indomethacin (3 mg/kg/day) reinforced hypoanabolism in the punch.

Thus, MIA-induced incapacity mimics human OA. As a predictable factor, handicap profile allows studies of early markers of OA detectable before installation of a marked incapacity. In addition, effects of treatments can be studied depending whether administered before or after the onset of this handicap. Further explorations on relationships between handicap and biochemical changes might lead to new pharmacological targets.

**Beneficial effect of polyclonal anti-Group II PLA2 antibodies in rat endotoxin shock**

Giuseppe Cirino, Maria Rosaria Bucci and Carla Cicala

Department of Experimental Pharmacology, via Domenico Montesano 49, 80131 Naples, Italy

Endotoxaemia leads to an increase in circulating levels of extracellular phospholipase A2 in animal models as well as in patients with septic shock (Vadas et al., 1992). We have tested rabbit polyclonal anti-PLA2 antibodies raised against human recombinant secreted non pancreatic PLA2 (Group II PLA2) and porcine pancreatic PLA2 (Group I PLA2) in rat endotoxin shock. Fall in mean arterial blood pressure induced by endotoxin (25 mg/kg i.v.; serotype 0127:B8) was 48 ± 3 mmHg (n = 13) while for anti-snp-PLA (0.5 mg/kg) and anti-porc-PLA2 (0.5 mg/kg) was 28 ± 3 mmHg (n = 5, p < 0.01) and 45 ± 10 mmHg (n = 4; ns). Anti-snp-PLA2 at the lower dose tested (0.1 mg/kg) was ineffective while the inhibition achieved with the higher dose (1 mg/kg) was similar to that obtained with the dose of 0.5 mg/kg. From a preliminary study was observed that maximal reduction in leukocyte platelet and haematocrit was obtained 4 h after LPS administration. Polyclonal anti-hnp-PLA2 antibodies at the dose of 0.5 mg/kg prevented the change in platelet count but not in leukocytes and haematocrit (figure; the line indicates platelet control value).

**Inhibitory effects of a mouse/human chimeric anti-TNFα antibody on in vitro immune function**

M. R. Dalesandro, C. S. Kinney, B. Frederick, B. Scallon and J. Ghrayeb

Pharmaceutical Division, Centocor, Inc., Malvern, PA, USA

Tumour necrosis factor (TNF) α, a cytokine produced by activated macrophages and lymphocytes, is a known mediator of both normal and chronic inflammation. Properties of TNFα including stimulation of IL-1 and collagenase are consistent with participation in the pathogenesis of rheumatoid arthritis (RA). Elevated levels of TNFα are found in the synovial fluid and on the membranes of activated CD4+ synoviocytes of RA patients. cA2, a mouse/human chimeric anti-TNFα antibody composed of the murine variable region joined to a human γ1 constant region, has shown clinical efficacy in the treatment of RA patients. Scatchard analyses reveal that cA2 γ1 and cA2 Fab bind human TNFα with equally high affinities and WEHI cytotoxicity assays show that they are equally efficient at inhibiting TNFα activity. In vitro assays showed that cA2 γ1 inhibited 60% of human peripheral blood cell proliferation in response to tetanus toxoid, mixed lymphocyte culture, or immobilized OKT3 antibody. The same molar concentration of cA2 Fab inhibited
proliferation by a maximum of 25%. Similarly, cA2 γ1 inhibited T-B cell collaboration measured by Ig synthesis more effectively than did the cA2 Fab. Since affinities and neutralization of cytotoxicity are equivalent for the cA2 γ1 and Fab fragments, we are currently pursuing the possibility that the greater immunomodulation observed with cA2 γ1 reflects a combined ability to neutralize soluble TNFα and to promote Fc-mediated death of activated cells expressing membrane-bound TNFα.

**Therapeutic intervention with mycobacterial 65 kDa heat-shock protein peptide 180-188 in adjuvant arthritis in Lewis rats**

Ulrich Feige and Jill Gasser

Ciba-Geigy, Department of Inflammation, R-1056.1.84, CH-4002, Basel, Switzerland

Adjuvant arthritis (AA) in Lewis rats is induced by an injection of mycobacteria in oil. AA is a T-cell mediated disease. Mycobacterial 65 kDa heat shock protein (hsp65) has been shown to be one of the antigens involved in the disease process. In fact, hsp65 or peptide 180–188 of hsp65 can be used to protect rats against an arthritogenic challenge with mycobacteria. However, therapy rather than prevention of disease appears to be the appropriate goal for intervention with disease in man. Therefore, we investigated whether peptide 180–188 also exhibits therapeutic effects in AA. We found that treatment of rats with 0.1–1.0 mg of peptide 180–188 in incomplete Freund’s adjuvant i.p. at days 9 and 10 after the arthritogenic challenge with mycobacteria in oil completely inhibited the onset of AA (no paw swelling, weight loss or radiographical changes were present). Even treatment at days 12 and 13, when clinical symptoms of disease (paw swelling and weight loss) were already apparent, was effective. The extent of bone destruction was reduced; individual radiographical scores at day 35 were 0, 0, 15, 72, 78 in peptide 180–188 treated rats and 44, 47, 57, 75, 101, 119 in AA control rats. The data indicate that immunological intervention with peptide 180–188 brings the disease process to a halt. This suggests that even in established disease the immune response is the major driving force of the disease process.

**Protection of human endothelial cells from the cytotoxic effects of activated granulocytes by Lazaroids and related lipophilic antioxidants**

William E. Fleming and Frank F. Sun

Hypersensitivity Diseases Research, Upjohn Labs, Kalamazoo, MI 49001, USA

The damaging of vascular endothelium by activated granulocytes is a typical feature of the inflammatory response. In vitro human granulocytes (neutrophils or eosinophils) activated by phorbol myristyl acetate (PMA) can injure and kill human umbilical vein endothelial cells (HUVECs). We incubated PMA activated human granulocytes with 51-chromium labelled HUVECs for 4 h and determined the extent of cell injury by measuring the release of radioactive into the medium. The results confirmed previous findings that granulocyte-induced cytotoxicity is iron and passage dependent with respect to the endothelial cells. Since it was postulated that the granulocyte derived reactive oxygen species were the predominant cytotoxic agents, we examined the effects of lipophilic antioxidants such as the lazaroid U-74006F, vitamin E, the vitamin E analogue U-78517F, the phenolic antioxidant nordihydroguaiaretic acid, the iron chelator desferroxamine, the antiinflammatory steroids and the water soluble antioxidant pyrrolidine dithiocarbamate (PDTC) as potential inhibitors of granulocyte induced endothelial cell cytotoxicity. As expected, the cytotoxicity was blocked by appropriate concentrations of lipophilic antioxidants. The results suggest that lipophilic antioxidants can protect against granulocyte-induced endothelial cell injury during inflammation.

**Zymosan induced arthritis in rats: pharmacological sensitivity**

P. Gégout, P. Gillet, D. Chevrier, C. Guingamp, B. Terlain and P. Netter

Department of Pharmacology, URA CNRS 1288, BP184, 54505 Nancy, France

Zymosan, a glycan derived from yeast cell wall is a phlogistic agent activating the alternative pathway of the complement system and inducing lysosomal enzyme release from PMNs and macrophages. Since zymosan-induced arthritis was mainly studied in mice, we have investigated its course in rats and assessed its inflammatory profile. Zymosan (2 mg) was injected (on day 0) in the right (histological and pharmacological assessment) or both knee joints (actimetry) Studies of patellar cartilage metabolism were assessed ex vivo through proteoglycan content and 35S incorporation. After injection of zymosan, swelling of right injected joints reached a maximum after 24 h and then decreased. Functional impairment was maximal the night following i.a. injection, and then decreased progressively. Histologically, the arthritis was characterized at day 14 by a chronic proliferative synovitis eroding cartilage. After an initial fall of chondrocyte proteoglycan synthesis peaking on day 2, occurred a transitory resumption on day 4. On day 20, anabolism of proteoglycans seemed normal, but proteoglycan content in articular patellae was significantly reduced (~18.6%).

Indomethacin (IND), when given orally from day-1, was effective on knee swelling only on day 1 and at high dose (3 mg/kg/day) while dexamethasone (DEX, 0.1 and 0.5 mg/kg/day) significantly reduced knee swelling through the first week. Histological score and locomotor activity were not improved by IND. In contrast, DEX...
Role of nitric oxide in the pathophysiology of pancreas inflammation

E. Gelpí, G. Hoter, D. Closa and J. Roselló-Catafau
Molecular Pathology Unit, Dpto. Bioanalítica Médica, Centro de Investigación y Desarrollo, CSIC, Barcelona, Spain

The relationship between nitric oxide production and prostanoid generation in two pancreas inflammatory process: ischaemia-reperfusion in pancreas transplantation and pancreatitis has been studied. For this purpose male Sprague-Dawley rats were subjected to pancreas transplantation, after 12 h preservation or acute pancreatitis induced by intraductal administration of 3.5% sodium taurocholate. In both cases, the effect of nitric oxide synthase inhibition with administration of N\textsuperscript{\textminus}nitro-l-arginine methyl ester (l-NAME) (10 mg/kg) was tested. The results show increases in 6-keto PGF\textsubscript{1α}, TXB\textsubscript{2} and PGE\textsubscript{2} in pancreatic tissue after transplantation or pancreatitis. In the case of transplantation, nitric oxide synthase inhibition reversed all the observed increases, suggesting that eicosanoid generation in this process would be mediated in part by a nitric oxide dependent mechanism. In contrast, in pancreatitis, nitric oxide synthase inhibition only reversed the increases in 6-keto PGF\textsubscript{1α} and TXB\textsubscript{2} levels, suggesting that endothelial and platelet eicosanoid generation are mediated through an nitric oxide-dependent mechanism, while acinar metabolites (PGE\textsubscript{2}) are generated in response to cell damage.

Antipyretic properties of paracetamol in normal rats and during various models of inflammation

P. Gillet, I. Cherkaoui, P. Gégout, F. Lapicque, E. Boccard, B. Terlain and P. Netter
Department de Pharmacologie, URA CNRS 1288, BP184, F54505, Nancy; Laboratoires UPSA, F92500 Rueil Malmaison, France

Paracetamol is a widely used antipyretic-analgesic drug, which inhibits cyclooxygenase (COX). Recent data have suggested the difference of sensitivity of COX, peripheral or central, constitutive (COX1) or inducible (COX2) to paracetamol. We have investigated the impact of paracetamol on body temperature (biotelemetry devices) in normal rats and during prostaglandin-dependent models of synovitis and fever. Paracetamol was injected i.p. (25; 50; 100; 200 mg/kg) through its prodrug, propacetamol (PRODAFALGAN).

In normal rat, body temperature and activity vary on a circadian basis. In this nocturnal animal, body temperature is highest at night and lowest during the day, mainly mediated by prostaglandins (PG). During day-time the highest dose of paracetamol (200 mg/kg) resulted in a slight decrease in body temperature with a maximal effect (Emax: -0.61°C) observed after a T\textsubscript{ma} of 1.75 h. During night-time, the 200 mg/kg dose only induced a fall in body temperature (Emax: -1.06°C; T\textsubscript{ma}: 1.5 h).

Injections of carrageenan in rat knees induced an acute fever (39°C) and nociceptive-induced hypomobility (60%). Paracetamol prevented transiently the febrile response and induced a dose-dependent fall in body temperature. With the 200 mg/kg dose E\textsubscript{max} (-1.27°C) was observed after a T\textsubscript{ma} of 2 h with an amplitude vs control batch of about -2.5°C. In contrast, paracetamol did not restore mobility in arthritic rats. Paracetamol administered in an established yeast-induced fever demonstrated dose-dependent antipyretic properties, maximal with the 200 mg/kg dose (Emax: -3.11°C; T\textsubscript{ma}: 2.5 h).

These results demonstrate hypothermic properties of high doses of paracetamol in normal rats, and antipyretic properties at therapeutic regimen in inflamed rats. The magnitude of maximal effect and duration of antipyretic properties appears related to the degree of activation of inducible COX. In addition central properties of paracetamol are more marked on central PG (fever) than on peripheral PG (synovitis with hypomobility).
Ultraviolet B-induced inflammatory cytokine production, in vivo: initial pharmacological characterization

D. E. Griswold and M. N. Tzimas
Department of Inflammation and Respiratory Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406-0939, USA

Tumour necrosis factor alpha (TNFα) and inflammatory cell infiltration following ultraviolet B (UVB) irradiation was studied. Balb/c mice were exposed to UVB irradiation (10–30 min) using a bank of six Westinghouse FS40 sunlamps (emitting a spectrum from 270–320 nm; 65% in UVB range). Energy output was 1067 μW/cm². TNFα was measured in tissue homogenates using an EIA. Myeloperoxidase (MPO) enzyme activity was used as a marker of neutrophil infiltration. A timecourse and dose-response of UVB revealed a delayed onset of response for both TNFα and MPO. Administration of naproxen (20 mg/kg, p.o.) appeared to exaggerate the response to UVB (122% increase of TNFα, p < 0.01; 15% increase of MPO, n.s.). In contrast, the phosphodiesterase inhibitor, rolipram (10 mg/kg, p.o.) significantly inhibited the release of TNFα (78%, p < 0.001) and the MPO response (61%, p < 0.001). This initial characterization of UVB-induced inflammation suggests a relationship between TNFα release and inflammatory cell infiltration and strong regulation of these events by cyclic AMP.

The modulation of adhesion molecule expression on endothelial cells by acteoside, a component of Stachis Sieboldii M.‘Q

K. Hayashi, T. Nagamatsu and Y. Suzuki
Meijo University, Nagoya 468, Japan

Recently, it has been indicated that adhesion of leukocytes to endothelial cells play the important role in both immune and inflammation response. The development of agent which is able to blunt the adhesion will be very beneficial to treat a lot of inflammatory diseases. Acteoside (ACT) inhibited the adhesion of neutrophils to cultured human umbilical vein endothelial cells (HUVEC) stimulated with tumour necrosis factor (TNFα) and up-regulation of ICAM-1 expression, but not endothelial leukocyte adhesion molecule-1 on HUVEC, with TNFα, interleukin-1β or phorbol myristate acetate. The inhibitory effect was not due to the toxicity of acteoside to HUVEC. On the other hand, ACT did not increase the amount of soluble ICAM-1 in the culture medium of HUVEC treated by TNFα, nor affected the interaction of ICAM-1 and BBIG-11 (anti-human ICAM-1 monoclonal antibody). The present data suggest that ACT is promising as an anti-inflammatory agent in a new category. Now, we are investigating the effect of ACT on ICAM-1 expression by thrombin which do not mediate the de novo synthesis of ICAM-1.

Effects of BIRM 270 on neutrophil functions

Departments of Biochemistry, Analytical Chemistry and Immunology, Boehringer Ingelheim Pharmaceuticals, Ridgefield City, CT, USA

Neutrophils are phagocytes which play a major role in host defence by emigrating from the blood compartment into sites of injury or infection where they release proinflammatory mediators and bactericidal agents. Recently, we identified a compound, BIRM 270, which potently inhibited the biosynthesis of LTβ, by calcium ionophore A23187-stimulated neutrophils. The compound was found to act by blocking the release of arachidonic acid. The present investigation was conducted to further define the effects of BIRM 270 on lipid mediator biosynthesis and various other neutrophil functions, some previously linked to arachidonate mobilization. Ionophore-stimulated arachidonate release and platelet activating factor biosynthesis (PAF), quantified by GC/MS, were inhibited similarly by BIRM 270, with > 90% inhibition achieved at 30 nM and half-maximal inhibition at about 14 nM. BIRM 270 did not affect any other neutrophil responses investigated, including: chemotaxis; Mac-1 expression (from mobilization of secretory vesicles); adherence to activated endothelium; lysozyme release (from degranulation of specific and azurophilic granules); lactoferrin release (from specific granules); or the generation of superoxide radical, as measured by the reduction of cytochrome C in the presence or absence of superoxide dismutase. We conclude that the actions of BIRM 270 are restricted to the biosynthesis of lipid mediators, and that arachidonate may not play an important second messenger role in other neutrophil functions, e.g. NADPH oxidase activation. In addition, our results provide pharmacological evidence that arachidonate mobilization and PAF biosynthesis may be coordinately linked.

Methyl arachidonyl fluorophosphonate, a potent irreversible cPLA2 inhibitor, blocks the mobilization of arachidonic acid in human platelets and neutrophils

Merck Frosst Centre for Therapeutic Research. P.O. Box 1005, Pointe-Claire, Dorval, Quebec, Canada, H9R 4P8

Methyl arachidonyl fluorophosphonate (MAFP) was designed as a cPLA2 inhibitor based on its preference for arachidonoyl moiety and the possible involvement of a serine residue in its active site. MAFP was found to be a potent time-dependent irreversible inhibitor of cPLA2. It inhibited cPLA2 stoichiometrically in vitro with an estimated koff of 0.9 s⁻¹ mol fraction⁻¹ on the 4 mol% DTEPM/Triton X-100 mixed micellar interface in the presence of calcium. Preincubation of cPLA2 with the reversible inhibitor AACOCF3 (arachidonoyl trifluoromethyl ketone) protected cPLA2 from MAFP, indicating AACOCF3 competes...
with MAFP. Whereas the human secreted 14-kDa phospholipase A\textsubscript{2} (sPLA\textsubscript{2}) was not inhibited by MAFP at concentration as high as 10 \textmu M. Thus, MAFP is a potent selective active-site directed inhibitor of cPLA\textsubscript{2}. MAFP was not an inhibitor for human 5-lipoxygenase when tested at 10 \textmu M (~30 \textmu M) in vitro; however, it inhibited the A23187-induced production of LT\textsubscript{B} in human neutrophils with an \textit{IC\textsubscript{50}} of ~0.1 \textmu M. Furthermore, MAFP blocked the A23187-induced AA release with an \textit{IC\textsubscript{50}} of 0.6 \textmu M in the presence of ETYA in human platelet. MAFP also inhibited A23187-induced 12-HETE (\textit{IC\textsubscript{50}} 0.6 \textmu M) and TxB\textsubscript{2} (\textit{IC\textsubscript{50}} 0.6 \mu M) production by platelets. In contrast, it did not inhibit 12-HETE and TxB\textsubscript{2} production in platelets when exogenous AA was used as a stimulus instead of A23187 (\textit{IC\textsubscript{50}} > 10 \textmu M). These results indicate that MAFP is a potent inhibitor capable of blocking the production of AA in neutrophils and platelets. cPLA\textsubscript{2} might be involved in A23187-induced mobilization of AA in neutrophils and platelets. The identical \textit{IC\textsubscript{50}} for inhibition of AA, TxB\textsubscript{2} and 12-HETE production in platelets suggest that TxB\textsubscript{2} and 12-HETE were derived from the same pool of AA.

**Platelet-derived growth factor (PDGF) and angiotensin II (A II) stimulate the mitogen-activated protein kinase cascade in renal mesangial cells**

A. Huwiler,\textsuperscript{1} S. Stabel,\textsuperscript{2} D. Fabbro\textsuperscript{2} and J. Pfeilschifter\textsuperscript{2}

\textsuperscript{1}Department of Pharmacology, Biozentrum, Switzerland; \textsuperscript{2}Max-Delbrück-Labor, Köln, Germany; \textsuperscript{2}Pharmaceuticals Division, Ciba-Geigy Ltd, Basel, Switzerland

Treatment of mesangial cells with PDGF and A II rapidly and dose-dependently stimulated mitogen-activated protein (MAP) kinase activity. Whereas stimulation with PDGF-BB caused a potent and sustained (for more than 30 min) phosphorylation and activation of p42\textsuperscript{\textit{MAPK}} and p44\textsuperscript{\textit{MAPK}} as well as of the upstream activators MAP kinase (MEK) and c-Raf, the effect of A II was less potent and peaked at 5–10 min and thereafter declined rapidly.

Down-regulation of protein kinase C (PKC-\textalpha) and -\delta isoenzymes by 4 h or 8 h treatment with phorbol 12-myristate 13-acetate (PMA) markedly inhibited PDGF-induced MAP kinase activation. Exposure to PMA for 24 h, a regimen that also depletes PKC-\epsilon, did not further reduce the level of activation by PDGF-BB, suggesting that both PKC-dependent and PKC-independent pathways are involved in PDGF-stimulated MAP kinase activation. Inhibition of PKC-\alpha and -\delta by PMA treatment for 24 h completely abolished A II-stimulated MAP kinase activity indicating that PKC-\epsilon substantially contributes to A II-stimulation of the MAP kinase pathway. Activators of protein kinase A, such as forskolin or N\textsuperscript{6}, 2\textquotesingle-O-dibutyryl-cAMP, attenuated PDGF- and A II-stimulated MAP kinase activation.

In summary, these results suggest that PDGF-BB and angiotensin II differ in their potency and duration of activating the MAP kinase cascade which may explain why PDGF-BB is a potent mitogen for mesangial cells whereas A II only triggers mesangial cell hypertrophy.

**Regulation and expression of prostaglandin G/H synthase in U937 cells**

J. J. Johnston and J. M. Trzaskos

The DuPont Merck Pharmaceutical Company, Inflammatory Diseases Research, P.O. Box 80400, Wilmington, DE 19880-0400, USA

Prostaglandin G/H synthase catalyses the rate limiting step in the production of prostaglandins from arachidonic acid. Inhibition of this enzyme by non-steroidal anti-inflammatory drugs (NSAIDs) has proven useful in the treatment of inflammatory conditions, however, mechanism-based GI and renal side effects are observed with most NSAIDs. Recently, an inducible form of PGHS has been discovered which is thought to be the major contributor to prostaglandin production during inflammation. In order to more completely define the contribution of this isofrom to inflammation, an understanding of the expression patterns of both PGHS genes is necessary. We have shown that numerous agents including serum, TPA, LPS, and TNF-\alpha can induce the expression of PGHS2 mRNA in fibroblasts while having minimal effects on PGHS1 expression. Using luciferase reporter gene constructs containing between 600 bp and 2 kb of the murine PGHS2 promoter region, luciferase activity can be induced by serum, while TPA, IL-1 and TNF-\alpha do not show an effect. When these reporter gene constructs are transfected into the monocytic U937 cell line they are responsive to TPA, showing a ten-fold increase in luciferase activity upon TPA stimulation. The inability of TPA to act through the promoter region in fibroblasts, coupled with the lack of clear AP-1 elements in this region of PGHS2 suggests that a monocyte specific factor may be responsible for the TPA response seen in U937 cells.

**Tetrahydrobiopterin is a limiting factor of nitric oxide generation in interleukin 1\beta-stimulated rat glomerular mesangial cells**

H. Mäbl and J. Pfeilschifter

Department of Pharmacology, Biozentrum, University of Basel, Basel, Switzerland

Treatment of mesangial cells with recombinant human interleukin 1\beta (IL-1\beta) triggers the expression of a macrophage-type of nitric oxide (NO) synthase and the subsequent increase of cellular concentration of cGMP and nitrite production. Tetrahydrobiopterin (BH\textsubscript{4}) is an essential cofactor for NO synthase and in the present study we investigated its impact on inducible NO synthesis in mesangial cells. Inhibition of GTP-cyclohydrolase I, the rate-limiting enzyme for BH\textsubscript{4} synthesis, with 2,4-diamino-6-hydroxy-pyridimline (DAHP) potently suppresses IL-1\beta-induced nitrite production and elevation of cellular cGMP levels. This inhibitory effect of DAHP is reversed by sepiapterin, which provided BH\textsubscript{4} via the pterin salvage pathway. Most importantly, sepiapterin dose-dependently augments IL-1\beta-stimulated NO synthesis, indicating that the availability of BH\textsubscript{4} limits the production of NO in cytokine-induced mesangial cells. N-acetylserotonin, an inhibitor of the BG4 synthetic enzyme sepiapterin...
reduces carrageenin-induced inflammation in rats, following either oral or parenteral administration. The compound also suppresses neutrophil emigration, possibly by blocking carbohydrate receptors on neutrophils or endothelial cells.

**Anti-inflammatory effect of BMS-181162, a PLA₂ inhibitor on two animal models of immune modulated inflammation**

X. Nair, L. Davern, C. Gabrel, P. Stanley and K. Tramposch

Dermatology Research, Buffalo, NY, USA

BMS-181162, a known inhibitor of PLA₂, is active against models of immune modulated inflammation, viz. The reverse passive arthus (RPA) model in rats and the delayed type hypersensitivity model in mice (DTH). The inflammatory response in the RPA model is precipitated by immune complex deposition, and complement-fixation, with PMN infiltration, and vascular permeability increase. Vascular permeability was measured by the accumulation of 125I-BSA in tissue and PMN infiltration was measured by myeloperoxidase (MPO) activity in the reaction site. BMS-181162 in a Tween 80/normal saline (0.5/99.5) vehicle was dosed i.p. 2 h before the administration of anti-BSA and BSA. Drug effect was measured 4 h post antigen/antibody administration. The DTH reaction in mice was induced by topical application of oxazolone. BMS-181162 was applied topically to the ear and PMN infiltration was measured by MPO activity. In the RPA model BMS-181162 caused a dose-dependent inhibition of vascular permeability and PMN infiltration. BMS-181162 at 50 mg/kg also significantly inhibited PGE₂ and LTB₄ increases. In the DTH assay BMS-181162 (2%) reduced PMN infiltration in the inflamed ear by 63%. It is significant that this PLA₂ inhibitor has demonstrated activity against two models of immune modulated inflammation following systemic and local administration. These results suggest the potential for developing agents that are active against both immune complex and cell mediated inflammation.

**Demonstration and characterization of anti-inflammatory activity in a carbohydrate (glycogen) extract of the New Zealand green-lipped mussel**

D. J. Ormrod, J. R. Dodd, R. Geddes† and T. E. Miller

Departments of Medicine and †Biochemistry, University of Auckland, Auckland, New Zealand

Many effective modern drugs were originally derived from plants or animals. In the past two decades there has been renewed interest in the search for pharmaceuticals in nature. The marine environment, with its huge and diverse reservoir of species, has received particular attention. Many potentially effective compounds have been isolated, but few have been used clinically. However, in cases where the parent organism can be economically harvested or farmed, nutraceuticals with apparent clinical benefits have reached the marketplace. Freeze-dried NZ green-lipped mussel (Perna canaliculus), marketed internationally as Seatone®, is one example (McFarlane Laboratories, Auckland, New Zealand). Laboratory and clinical investigations have shown that this preparation inhibits experimentally induced inflammation and may provide symptomatic relief in individuals with arthritis. We have studied the mollusc in an effort to identify the active moiety. Our most recent results demonstrate that the activity resides in a glycogen fraction of the mussel. The extract effectively reduces carrageenin-induced inflammation in rats, following either oral or parenteral administration. The compound also suppresses neutrophil emigration, possibly by blocking carbohydrate receptors on neutrophils or endothelial cells.

**Transcriptional activation of lipocortin-1**

L. Parente, F. Russo-Marie and E. Solito

I.R.I.S. Siena, Italy and I.C.G.M. Inserm U332, Paris, France

Lipocortin-1 (annexin-1) is a member of a family of proteins with calcium and phospholipid binding properties which is a putative mediator of the anti-inflammatory action of glucocorticoids. Since controversial data have been reported on the capability of steroids of inducing the synthesis of lipocortin-1 we have studied the promoter region of 880 bp of the protein gene which contains the first not translated exon and the first intron. Different cell lines (HeLa, U-937, A-549) were transfected with a plasmid containing the luciferase reporter gene under the control of the lipocortin-1 promoter. Transient expression of the reporter gene was enhanced by phorbol 12-myristate 13-acetate (PMA, 10 nM), dexamethasone (DEX, 1 μM), and IL-1 (500 pg/ml). Interestingly the stimulatory effect of PMA was counteracted by a short incubation (2 h) with DEX while it was potentiated by a long treatment (16 h) with the glucocorticoid. These results indicate that glucocorticoids are indeed able to enhance gene expression of lipocortin-1. The inhibitory effect of 2 h DEX on PMA-stimulated gene expression may be explained by interference with AP-1 activity, which is induced by PMA, by direct interaction or by DNA binding. The synergistic effect of 16 h DEX may be due to phosphorylation by PMA of transcription factors interacting with the glucocorticoid receptor.
Ascites is a complication of portal hypertension and of peritoneal cancer. Spontaneous bacterial peritonitis (SBP) is a serious complication in these patients. We measured soluble ICAM-1 (sICAM-1), several cytokines (interleukin-1β, IL-6 and tumour necrosis factor-α (TNF-α)) and several eicosanoids (LTB₄, PGE₂, TXB₂ and 6kPGF₁α) in ascites and serum from patients (54) with cirrhosis (alcoholic, viral, PBC and other causes), peritoneal cancer (26) and SBP (10) to determine whether the pattern of mediators found could be of diagnostic and/or prognostic aid.

Ascitic sICAM-1, the cytokine IL-6 and the eicosanoids LTB₄ and PGE₂ were significantly elevated in peritoneal cancer compared with portal hypertension. Ascitic IL-6 was significantly elevated in SBP compared with portal hypertension and peritoneal cancer. There was a positive correlation (p < 0.001) between serum and ascites sICAM-1 and IL-6, TNFα and endotoxin, and PGE₂, TXB₂ and 6kPGF₁α in ascites and serum from patients (54) with cirrhosis (alcoholic, viral, PBC and other causes), peritoneal cancer (26) and SBP (10) to determine whether the pattern of mediators found could be of diagnostic and/or prognostic aid.

Conclusion: There are significant differences in concentrations of inflammatory products in ascites due to different disorders, but none of these mediators seems to be useful as a diagnostic parameter as there is an overlap between all the levels of these mediators in ascites. SICAM-1 and IL-6 could be of prognostic aid for patients with SBP, as the episodes of infection correlate with the levels of these mediators.

**Pharmacologic efficacy of LY293111, a potent orally active leukotriene B₄ (LTB₄) receptor antagonist, in humans**

S. M. Spaeteb,1 P. Marder,1 L. L. Froelich,1 B. H. Petersen,1 T. W. Croghan,1 R. A. Lucas,1 T. Tanner1 and J. S. Sawyer1

1Lilly Research Laboratories, Indianapolis, IN, USA; 2Lilly Research Centre, Windlesham, UK; 3Simbec Research Ltd, Merther Tydfil, South Wales, UK

A common element of inflammation is the accumulation and activation of leukocytes at inflammatory foci. These events are directed by biological mediators, including LTB₄. Among the various physiological changes that neutrophils (PMNs) undergo during activation is an increase in the number of the adhesion receptors, CD11b/CD18, on the cell surface as measured by flow cytometry. Pre-incubation of human PMNs, either isolated or residing in whole blood with LY293111 prevents the subsequent upregulation of CD11b/CD18, on the cell surface as measured by flow cytometry. Pre-incubation of human PMNs, either isolated or residing in whole blood with LTB₄ induced CD11b receptor upregulation was measured over time for each subject. There was marked inhibition (>73%) of LTB₄-induced CD11b expression on whole blood PMNs from volunteers receiving either 60 or 120 mg t.i.d., or 200 mg b.i.d. 8–12 h after dosing. This inhibition was reversible upon termination of dosing. Additional studies demonstrated the selectivity of LY293111 utilizing an alternative agonist, fMLP, in these same subjects following the high dose of LY293111. We conclude that LY293111 has potent, selective pharmacologic activity in man. These observations could provide the basis for novel therapeutic treatments of human inflammatory diseases.

**Activation of human eosinophils by cytokines: differential expression of adhesion/activation markers and potentiation of survival by chemokines**


Upjohn Laboratories, Kalamazoo, MI 49001, USA

We have examined by flow cytometric analysis the differential induction of a panel of adhesion/activation markers
Abstracts

on normal human peripheral blood eosinophils by IL-3, IL-5 and GM-CSF after 18 h and 48 h of in vitro culture. The expression of CD11a, CD11b, CD11c and CD18 were increased 18 h and 48 h after activation by all three cytokines at 10 ng/ml. Of the cytokines tested, IL-3 had the most pronounced effect when compared with IL-5 and GM-CSF. CD49b, CD49e and CD49f were found on very few of the inactivated eosinophils, but CD49d and CD29 were present on >90% of these cells. Expression of the β1 integrins remained unchanged even after 48 h of incubation. All of the eosinophils expressed CD44, CD58 (LFA-3), CD45RO, CD67, CD9, CD63 and CD31 but the mean channel fluorescence (MCF) of these antigens (except for CD31 and CD63) was elevated by IL-3, IL-5 and GM-CSF. The percentage of eosinophils which expressed CD25, CD69 and CD54, and the MCF were both increased by these cytokines. Conversely, the expression of L-selectin was dramatically reduced by cytokine treatment (IL-3>IL-5=GM-CSF). RANTES, MCP-1, or MIP-1α/β (all at 20 ng/ml) did not alter the expression of any of the eosinophil cell surface antigens. Furthermore, these chemokines did not potentiate the effect of IL-3 or IL-5. RANTES, MCP-1, or MIP-1α/β alone did not prolong the survival of eosinophils even when the cells were placed on laminin, Collagen Type I or IV, and fibronectin-coated plates. However, the prolongation of eosinophil survival by IL-3 was enhanced by the addition of chemokines. Potential cytokine and chemokine interactions may contribute to the pathophysiology of eosinophilic inflammation.

Involvement of CD36 in acute lung inflammation and injury in IgG immune complex-mediated rat lung injury

Z.-J. Yang, C. Black and B. Beaubien
Allelix Biopharmaceuticals Inc., Mississauga, Ontario, Canada L4V 1V7

CD36 is a cell surface receptor for thrombospondin (TSP), and has been determined to mediate significant effects of TSP on cell adhesion and platelet aggregation. Since elevated TSP levels have also been demonstrated in inflammatory sites (J Immunol 1989; 143: 1969-73), we investigated the role of CD36 in acute lung inflammation using a rat model of IgG immune complex-induced alveolitis (J Clin Invest 1974; 54: 349–357). To generate the immune response, rats were given bovine serum albumin (BSA) intravenously and anti-BSA IgG intratracheally. Four hours later, the following parameters were measured: total cell numbers, cell differentials, haemoglobin concentrations in bronchoalveolar lavage (BAL), plasma extravasation in BAL, and plasma exudation in perfused lungs. Monoclonal antibodies (Mabs) directed against human CD36 (OKM5 and MCA722) induced a dose-dependent inhibition of airway leukocyte infiltration, hemorrhage and plasma exudation. The two Mabs were approximately equipotent. Intratracheal administration of the same dose of Mab was more effective than an intravenous dose. Isotype control immunoglobulins had no effect. These findings demonstrate for the first time that CD36 is involved in lung damage in this model.
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