Immunology 1987 **60** 203–207

Purified interleukin-1 (IL-1) from human monocytes stimulates acute-phase protein synthesis by rodent hepatocytes *in vitro*

J. GAULDIE,*† D.N. SAUDER,† K. P. W. J. McADAM‡§ & C. A. DINARELLO‡ *Departments of *Pathology and †Medicine, McMaster University, Hamilton, Ontario, Canada and ‡Departments of Medicine and Pediatrics, Division of Experimental Medicine, Tufts University, Boston, Massachusetts, U.S.A.*

Accepted for publication 17 October 1986

SUMMARY

A universal component of inflammation is the increased synthesis of a series of plasma proteins (acute-phase proteins) by the liver. The postulated messenger of acute-phase protein induction is released by leucocytes at the site of inflammation and has been shown to co-purify with endogenous pyrogen or lymphocyte-activating factor. Interleukin-1, molecular weight 17,000, pI 6·8–7·2, was purified to homogeneity from adherent human blood monocytes by a combination of affinity chromatography, gel filtration and isoelectric focusing. We examined the direct effect of pure IL-1 on the induction of acute-phase protein synthesis *in vitro* using rat and mouse hepatocytes. IL-1 caused significant increased synthesis of α1-acid glycoprotein and smaller increases in the synthesis of other acute-phase proteins, and significant decreased synthesis of albumin. The pattern of induction of acute-phase proteins differs from that seen with a separate 30,000 molecular weight hepatocyte-stimulating factor from human monocytes described previously. We conclude that human IL-1 is one of the mediators responsible for the acute-phase protein response of the liver in inflammation and can directly cause stimulation of specific gene expression in normal hepatocytes.

INTRODUCTION

Recent evidence indicates that polypeptide cytokines released by monocytes/macrophages and a limited number of other cells interact specifically with the liver to initiate acute-phase protein synthesis. Interleukin-1 (IL-1), previously referred to as lymphocyte-activating factor (LAF), a 17,000 molecular weight (MW) family of cytokines, in various stages of purity can initiate plasma protein changes *in vivo*, and there are limited reports of hepatocyte responses to these cytokines *in vitro*. Our recent work (Koj *et al.*, 1984, 1985) and that of others (Ritchie & Fuller, 1983; Baumann *et al.*, 1984; Woloski & Fuller, 1985) show the presence in the same monocyte supernatants of a second larger cytokine (MW 30,000, pI 5), hepatocyte-stimulating factor (HSF), which induces a full spectrum of acute-phase proteins in isolated hepatocyte cultures.

In order to determine whether IL-1 has the same *in vitro* effect as HSF, we chose to investigate whether highly purified homogeneous human monocyte-derived IL-1 (MW 17,000, pI 7), shown to be active *in vivo* with numerous assays of IL-1 activity, could initiate a similar hepatocyte acute-phase protein response *in vitro* using hepatocyte culture conditions in which the larger HSF is known to be active. This report shows that IL-1 (pI 7) can induce the acute-phase response but that the spectrum of acute-phase proteins induced by this molecule is restricted and differs from that induced by the larger hepatocyte-stimulating factor. These data confirm and expand the preliminary findings first reported at the conference on the actions of IL-1 in 1985 (Gauldie *et al.*, 1985).

§Present address: London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.

Correspondence: Dr J. Gauldie, Dept. of Pathology, McMaster University, 1200 Main Street W., Hamilton, Ontario L8N 3Z5, Canada.

MATERIALS AND METHODS

Purified human IL-1

Human IL-1, MW 17,000, pI 7, was purified from peripheral blood monocyte supernatants as described in detail elsewhere (Dinarello *et al.*, 1985). Briefly, monocytes were isolated, pulsed with ^{35}S-methionine and stimulated with *Staphylococcus albus* toxin. The supernatant was subjected to affinity chromatography using an antibody to human endogenous pyrogen, and to molecular sieve chromatography on Sephadex G50-fine (170 × 2·4 cm). Fractions were tested for lymphocyte-activating factor activity using the cloned mouse T-cell line D10.G4.1 assay (Auron *et al.*, 1984), and active fractions were pooled (MW 15,000–20,000). Preparations were then subjected to chromatofocusing and the fractions tested for pH and LAF activity.

In vitro hepatocyte stimulation (HSF)

Rat hepatocyte stimulation of the production of acute-phase proteins was assayed as previously described (Koj *et al.*, 1985). Briefly, rat hepatocytes were isolated by collagenase digestion via portal vein perfusion, and cultured in medium containing 5% fetal calf serum for 72 hr in T24 Linbro plates. Samples were dialysed against phosphate-buffered saline and 50 μl of each sample were added at the start of culture and at each 24 hr with medium changes (200 μl). The supernatants derived from cells removed at 48–72 hr were tested for acute-phase protein content by rocket immunoelectrophoresis. Analysis of these supernatants detects *de novo* synthesis of these proteins (Koj *et al.*, 1984). The specific antisera used to detect albumin, α1-acid glycoprotein, α2-acute phase macroglobulin and fibrinogen, and standard preparations of antigens, were as previously described (Koj *et al.*, 1984). LPS (10 μg/ml) was included in two wells, and phosphate-buffered saline in a further two wells, as controls and showed no significant stimulation of synthesis of acute-phase proteins. A sample of peripheral blood monocyte supernatant with known HSF and LAF activity was included as a positive control in each assay. This material causes significant synthesis of most acute-phase proteins by these cells. Results are expressed as the ratio of the concentrations of secreted specific acute-phase proteins to albumin, which has previously been shown to be a sensitive indication of hepatocyte stimulation (Koj *et al.*, 1985).

Mouse hepatocyte stimulation of serum amyloid A protein synthesis was assayed as previously described (Selinger *et al.*, 1980). Briefly, mouse hepatocytes, derived from endotoxin non-responder C3H/HeJ mice, were isolated by collagenase digestion via portal vein perfusion, and cultured using serum free medium for 48 hr in T24 Linbro plates. Samples of cytokine were dialysed against medium, and 200 μl were added to a total volume of 2 ml medium and the cells cultured for a further 24 hr. Supernatants were then tested for the presence of serum amyloid A protein by a sensitive enzyme immunoassay.

Lymphocyte activation assay (LAF)

Measurement of LAF activity was carried out using either the D10 cloned murine T-cell line assay (Dinarello *et al.*, 1985) or the PHA-costimulator C3H/HeJ mouse thymocyte assay as previously described (Koj *et al.*, 1984).

RESULTS

Three separate purifications and chromatofocusing column fractionations were examined for *in vitro* hepatocyte stimulation. Figure 1a shows the typical distribution of ^{35}S-methionine-labelled protein with the peak of activity emerging at fraction 30 corresponding to a pI of 6·8. Figure 1b shows the LAF activity of these fractions assayed at 1/100 dilution in the D10 mouse thymocyte assay. Peak LAF activity is shown emerging at fraction 30. Silver-staining and autoradiography of SDS gels of each fraction show only a single band of protein in fractions 28–32 with a molecular weight of 17,000–19,000 (Auron *et al.*, 1987).

Rat hepatocytes

Figure 1c shows that the fractions caused small but significant stimulation of α2-macroglobulin (α2M) synthesis with the peak

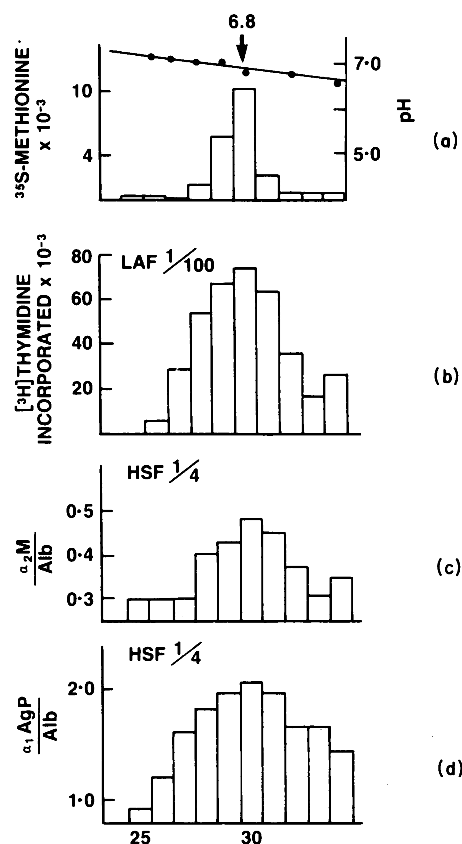

Figure 1. Isoelectric focusing separation of ^{35}S-methionine-labelled human peripheral blood monocyte derived interleukin-1. (a) Distribution of ^{35}S counts and pH of chromatofocusing fractions. (b) Lymphocyte activation factor activities of column fractions determined with the D10 cloned lymphocyte assay. Fractions were assayed at 1/100 dilution. (c) Ratio of α2-macroglobulin and albumin production by rat hepatocytes-incubated with fractions diluted 1/4. (d) Ratio of α1-acid glycoprotein and albumin production by rat hepatocytes incubated with fractions diluted 1/4.

of activity corresponding to the peak of LAF activity, while Fig. 1d shows that these same fractions caused a more marked stimulation of α1-acid glycoprotein (α1AgP) production with the major peak of activity at fraction 30. Fibrinogen and α1 acute-phase globulin were marginally stimulated (maximum 15–25%) in a similar manner to α2-macroglobulin.

Figure 2a shows that the same cytokine both stimulates acute-phase protein synthesis and inhibits albumin synthesis. The fractions show an identical distribution of LAF activity in both types of thymocyte proliferation assays (Fig. 2b).

Two other purifications showed similar results. The fractions exhibiting the highest LAF, MW 17,000 and pI approximately 7, caused maximum hepatocyte synthesis of rat α1-acid glycoprotein synthesis, with small but significant stimulation in α2-macroglobulin synthesis, and marginal stimulation in α1 acute-phase globulin and fibrinogen synthesis, similar to that shown in Fig. 1.

Mouse hepatocytes

In contradistinction to considerable stimulation of synthesis of

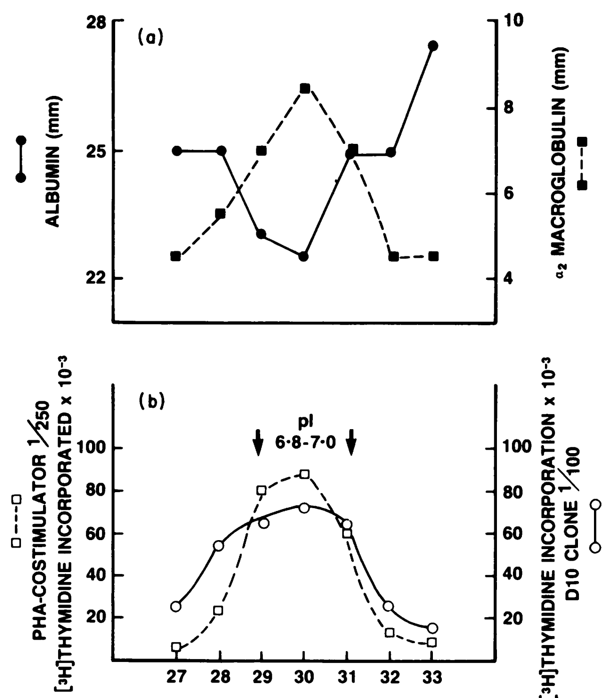

Figure 2. Examination of hepatocyte stimulation and lymphocyte activation activities of chromatofocusing fractions 27–33. (a) Rocket immunoelectrophoresis peak heights of supernatants from rat hepatocytes incubated with column fractions 27–33: (●——●) albumin; (■——■) α2-macroglobulin production. (b) Lymphocyte activation factor assay of column fractions in D10 cloned lymphocyte assay (○——○) and in C3H/HeJ mouse thymocyte PHA costimulator assay (□——□).

some acute-phase proteins in rat hepatocytes, when these same fractions were tested in the mouse hepatocyte assay, only minor elevation (10–15%) of SAA production was caused by active fractions 27–33, despite evidence to suggest that the same fractions induced considerable SAA synthesis (plasma levels of up to 40–50 μg/ml) in C3H/HeJ mice when administered i.v. 12 hr previously (Auron *et al.*, 1987). Assays of albumin production of mouse hepatocytes were unchanged, showing that the lack of distinctive synthesis was not due to toxic effects of the preparations.

DISCUSSION

Liver response to injury is one of the many facets of the acute-phase reaction, which includes a febrile response, leukocytosis and altered cellular metabolism of numerous tissues (Koj, 1974). The liver responds early to trauma with increased uptake of amino acids as well as iron and zinc. This is followed by intracellular increases in certain enzymes, such as sialyltransferase (Kaplan *et al.*, 1983), and increased transcription of mRNA for various proteins such as α1-acid glycoprotein (Baumann *et al.*, 1984; Ricca *et al.*, 1981; Northemann *et al.*, 1983). Subsequent to these events, there is a characteristic change in the plasma levels of a series of proteins synthesized by the liver, commonly known as the acute-phase reactants.

There is considerable species variation in the nature of specific plasma protein changes seen in inflammation (Gauldie, Lamontagne & Stadnyk, 1985). In man the most dramatic changes in plasma levels are seen in C-reactive protein, serum amyloid A protein and α1-acid glycoprotein (orosomucoid); in the rat the most significant plasma changes occur in acute-phase α2-macroglobulin, a broad-spectrum proteinase inhibitor, α1 acute-phase globulin, a cysteine-proteinase inhibitor, and α1-acid glycoprotein; in the mouse major plasma changes occur in serum amyloid A protein, serum amyloid P component, and α1-acid glycoprotein. Other proteins, such as fibrinogen, α1-proteinase inhibitor (α1-anti-trypsin), α1-anti-chymotrypsin, haptoglobin and ceruloplasmin show intermediate but significant plasma increases, while albumin, transferrin and α- and β-lipoproteins show a coincidental decrease in plasma levels in most species. Several recent reviews have summarized the current views on the nature and control of the acute-phase response (Koj, 1974, 1984; Kushner, 1982; Pepys & Baltz, 1983; Gauldie *et al.*, 1985; Gordon & Koj, 1985).

In the process of inflammation, the role of IL-1 as the activator of most, if not all, metabolic changes associated with host response seems attractive (Dinarello, 1984a, b, c, 1985a; Lachman, 1983; Kampschmidt, 1984). Synthesis of acute-phase proteins constitutes a significant *in vivo* metabolic change in hepatocyte function during inflammation. Previous reports have indicated that the mediator responsible for the initiation of this change is known variously as leucocyte endogenous mediator, endogenous pyrogen, lymphocyte-activating factor and interleukin-1 (Selinger *et al.*, 1980; Kampschmidt, 1978; Hooper *et al.*, 1981; Sipe *et al.*, 1979, 1982; McAdam *et al.*, 1982; Le, Muller & Mortensen, 1982; Dinarello, 1984a, b). The majority of these reports used either crude monocyte/macrophage supernatant, or fractions thereof with varying purity. The activity has been shown both *in vivo* and *in vitro*.

However, previous work in one of our laboratories (JG) demonstrated the presence of a 30,000 MW protein in LPS-stimulated human peripheral blood monocyte supernatants. This cytokine, hepatocyte-stimulating factor (HSF), is a major stimulator of *in vitro* hepatocyte synthesis of many acute-phase proteins (Koj *et al.*, 1984, 1985), and the activity is clearly separable from LAF activity with an apparent pI of 5–5·2. Others have reported similar findings for HSF (Ritchie & Fuller, 1983; Baumann *et al.*, 1984). Woloski & Fuller (1985) recently reported the release of this 30,000 MW factor from leukaemic cell lines of the monocytic series, and in addition they suggested that IL-1 (17,000 MW) had no activity on *in vitro* rat hepatocyte synthesis of fibrinogen.

Taken together, these results could suggest that previous reports of induction of acute-phase proteins by preparations of IL-1 could have been due to the presence of various amounts of 30,000 MW hepatocyte-stimulating factor. It was therefore important to examine several preparations of highly purified human monocyte-derived IL-1, selected for LAF activity and 17,000 MW, for the initiation of the acute-phase protein response, particularly using the same rat hepatocyte system with which we had demonstrated the existence of the 30,000 MW hepatocyte-stimulating factor.

The purification scheme used to prepare the IL-1 leads to a single homogeneous polypeptide with pI 6·8, and fluorography (^{35}S-methionine) of SDS–acrylamide analysis of the chromatofocusing column shows only a single band of activity at 17,000 MW. No other proteins are seen by either protein stain or fluorography (Dinarello *et al.*, 1985).

An examination of Fig. 1 shows that the chromatofocusing profile of activity for ^{35}S-methionine content and LAF activity is superimposable on that of rat hepatocyte stimulation (both acute-phase protein induction and albumin synthesis suppression, Fig. 2). The same was true for the other two purifications (data not shown). In a related series of studies, the same column fractions were assayed for other IL-1-related activities. The same coincidence of maximum activities was noted for LAF, pyrogenic activity, fibroblast prostaglandin PGE_2 induction, and *in vivo* serum amyloid A (SAA) induction (Dinarello *et al.*, 1985; Auron *et al.*, 1987).

Despite this evidence for *in vitro* rat hepatocyte stimulation of α1-acid glycoprotein synthesis and considerable *in vivo* mouse SAA induction (Auron *et al.*, 1987), the purified IL-1 preparation caused only minimal stimulation of SAA synthesis by mouse hepatocytes *in vitro*. In addition, we saw only minor changes in α2-macroglobulin, fibrinogen and α1 acute-phase globulin synthesis by rat hepatocytes, proteins that normally exhibit marked changes *in vivo* and which show marked stimulation by HSF-containing monocyte supernatants. An explanation of this anomalous behaviour can be taken from an examination of the phenotype of primary rat hepatocytes in culture. We have previously shown (Koj *et al.*, 1984) that normal rat hepatocytes appear to be in a state of stimulation, probably induced by the isolation procedure and steroid incubation, and synthesize plasma proteins *in vitro* at a rate more consistent with plasma concentrations seen *in vivo* during the acute-phase response (the level of α2-macroglobulin synthesized by rat hepatocytes *in vitro* is in vast excess of that expected from the low or undetectable level in normal plasma, the same phenomenon being found for α1-acid glycoprotein and serum amyloid A in the mouse). The cells may not be able to respond fully to a further challenge with stimulator, and this would explain the much less spectacular change than expected caused by cytokine *in vitro* from results seen during inflammation *in vivo* (Koj *et al.*, 1982; Schreiber *et al.*, 1982). Recently, work by Ramadori *et al.* (1985), using similarly purified as well as rDNA-derived murine IL-1, demonstrated significant stimulation of SAA production by isolated mouse hepatocytes. In addition, rDNA-derived IL-1 causes *in vivo* acute-phase protein induction in mice (Dinarello, 1985b). While these results appear to contrast with our mouse results, they are confirmed by our findings in rat hepatocytes, and the discrepancy may simply represent varied levels of basal stimulation obtained in different mouse hepatocyte preparations in the different laboratories.

Were we to examine the changes in only one of the acute-phase proteins synthesized by rat hepatocytes, such as fibrinogen or α1 acute-phase globulin or haptoglobin, caused by this purified IL-1, we would be hard-pressed to demonstrate significant stimulation. This is indeed the case for SAA in the mouse seen here and would be consistent with the conclusions reached by Woloski & Fuller (1985). Examination of Fig. 2a demonstrates, however, that IL-1 exerts appropriate positive and negative effects on proteins synthesized by rat hepatocytes, and shows that purified human IL-1 causes a consistent but restricted *in vitro* stimulation of rat acute-phase proteins, with the major change being seen in α1-acid glycoprotein.

In a separate series of experiments to be reported elsewhere, we have shown that other purified monocyte-derived factors, one causing interferon induction and referred to as human '22K factor', which is related to and probably is an IL-1 molecule (Van Damme *et al.*, 1985), and others purified from porcine monocytes and referred to as catabolin (IL-1) (Saklatvala *et al.*, 1984), cause similar marked stimulation of α1-acid glycoprotein synthesis and lesser stimulation of the other acute-phase reactants along with decreased synthesis of albumin by rat hepatocytes.

These results indicate that IL-1 purified from human monocytes with molecular weight of 17,000 and with pI of 6·8–7·2 causes *in vitro* rat hepatocyte stimulation and is therefore one of the molecules responsible for the increased synthesis of acute-phase reactants during inflammation. The relationship of IL-1 (MW 17,000, pI 5 and 7) to HSF (MW 30,000, pI 5) will require examination of highly purified 30,000 MW material. It is possible that the 30,000 MW HSF is a precursor molecule of IL-1 that may lack LAF activity. It is also possible that the molecules bear no relationship and may act through separate hepatocyte receptors. With purified and/or cloned IL-1 now becoming available (Dinarello *et al.*, 1985; Auron *et al.*, 1984; Lomedico *et al.*, 1984; Kronheim *et al.*, 1985; March *et al.*, 1985), receptors can be identified. These results describe activities *in vitro*. The direct role of IL-1 or other cytokines *in vivo* on liver cell function has not been addressed here but, with the availability of rDNA material, such important studies can be undertaken.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council of Canada and the National Institutes of Health, U.S.A. The assistance of J. Schroeder and J. Hickey is gratefully acknowledged, as is the help of G. LoPreste.

REFERENCES

Auron P.E., Warner S.J.C., Webb A.C., Cannon J.G., Bernheim H.A., McAdam K.P.W.J., Rosenwasser L.J., LoPreste G., Mucci S.F. & Dinarello C.A. (1987) Studies on the molecular nature of human interleukin 1. *J. Immunol.* (in press).

Auron P.E., Webb A.C., Rosenwasser L.J., Mucci S.F., Rich A., Wolfe S.M. & Dinarello C.A. (1984) Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. natl. Acad. Sci. U.S.A.* **81,** 7907.

Baumann H., Jahreis G.P., Sauder D.N. & Koj A. (1984) Human keratinocytes and monocytes release factors which regulate the synthesis of major acute phase proteins in hepatic cells from man, rat and mouse. *J. biol. Chem.* **259,** 7331.

Dinarello C.A. (1984a) Interleukin 1. *Rev. infect. Dis.* **6,** 51.

Dinarello C.A. (1984b) Interleukin-1 and the pathogenesis of the acute-phase response. *New Eng. J. Med.* **311,** 1413.

Dinarello C.A. (1984c) Interleukin 1 as mediator of the acute phase response. *Surv. Immunol. Res.* **3,** 29.

Dinarello C.A. (1985a) An update on human Interleukin-1: from molecular biology to clinical relevance. *J. clin. Immunol.* **5,** 287.

Dinarello C.A. (1985b) New perspectives in the study of human interleukin 1: contribution from molecular biology. In: *The Physiologic, Metabolic and Immunologic Actions of Interleukin-1* (eds M.J. Kluger, J.J. Oppenheim and M.C. Powanda), p. 439. A. R. Liss, New York.

Dinarello C.A., Bernheim H.A., Cannon J.G., LoPreste G., Warner S.J.C., Webb A.C. & Auron P.E. (1985) Purified, ^{35}S-MET, ^{3}H-LEU-labelled human monocyte interleukin-1 (IL-1) with endogenous pyrogen activity. *Br. J. Rheumatol.* **24** (Supp. 1), 56.

Gauldie J., Lamontagne L. & Stadnyk A.W. (1985) Acute phase response in infectious disease. *Surv. Synth. Pathol. Res.* **4,** 126.

GAULDIE J., SAUDER D.N., MCADAM K.P.W.J. & DINARELLO C.A. (1985) Purified human monocyte IL-1 stimulates hepatocyte secretion of multiple acute phase proteins *in vitro*. In: *The Physiologic, Metabolic and Immunologic Actions of Interleukin-1* (eds M.J. Kluger, J.J. Oppenheim and M.C. Powanda), p. 221. A. R. Liss, New York.

GORDON A.H. & KOJ A. (eds) (1985) The acute phase response to injury and infection. *Research Monographs in Cell and Tissue Physiology*, Vol. 10. Elsevier Scientific Publishers, Amsterdam.

HOOPER D.C., STEER C.J., DINARELLO C.A. & PEACOCK A.C. (1981) Haptoglobin and albumin synthesis in isolated rat hepatocytes. Response to potential mediators of the acute-phase reaction. *Biochim. biophys. Acta*, **653**, 118.

KAMPSCHMIDT R.F. (1978) Leukocytic endogenous mediator. *J. Reticuloendothel Soc.* **23**, 287.

KAMPSCHMIDT R.F. (1984) The numerous postulated biological manifestations of interleukin-1. *J. Leukocyte Biol.* **36**, 341.

KAPLAN H.A., WOLOSKI B.M.R.N.J., HELLMAN M. & JAMIESON J.C. (1983) Studies on the effect of inflammation on rat liver and serum sialy-transferase. *J. biol. Chem.* **19**, 11505.

KOJ A. (1974) Acute phase reactants. In: *Structure and Function of Plasma Proteins* (ed. A. C. Allison), p. 73. Plenum Press, New York.

KOJ A. (1984) Metabolic studies of acute-phase proteins. In: *Pathophysiology of Plasma Protein Metabolism* (ed. E. Mariani), p. 221. MacMillan, New York.

KOJ A., DUBIN A., KASPERCZYK H., BERETA J. & GORDON A.H. (1982) Changes in the blood level and affinity to concanavalin A of rat plasma glycoproteins during acute inflammation and hepatoma growth. *Biochem. J.* **206**, 545.

KOJ A., GAULDIE J., REGOECZI E., SAUDER D.N. & SWEENEY G.D. (1984) The acute-phase response of cultured rat hepatocytes. System characterization and the effect of human cytokines. *Biochem. J.* **224**, 505.

KOJ A., GAULDIE J., SWEENEY G.D., REGOECZI E. & SAUDER D.N. (1985) A simple bioassay for monocyte-derived hepatocyte stimulating factor: increased synthesis of alpha-2-macroglobulin and reduced synthesis of albumin by cultured rat hepatocyte. *J. immunol. Meth.* **76**, 317.

KRONHEIM S.R., MARCH C.J., ERB S.K., CONLON P.J., MOCHIZUKI D.Y. & HOPP T.P. (1985) Human interleukin 1. *J. exp. Med.* **161**, 490.

KUSHNER I. (1982) The phenomenon of the acute phàse response. *Ann. N.Y. Acad. Sci.* **389**, 39.

LACHMAN L.B. (1983) Human interleukin 1: purification and properties. *Fed. Proc.* **42**, 2639.

LE P., MULLER M.T. & MORTENSEN R.F. (1982) Acute phase reactants of mice. I. Isolation of serum amyloid P-component (SAP) and its induction by a monokine. *J. Immunol.* **129**, 665.

LOMEDICO P.T., GUBLER U., HELLMANN C.P., DUKOVICH M., GIRI J.G., PAN Y.-C.E., COLLIER K., SEMIONOW R., CHUA A.O. & MIZEL S.B. (1984) Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli*. *Nature (Lond.)*, **312**, 458.

MARCH C.J., MOSLEY B., LARSEN A., CERRETTI D.P., BRAEDT G., PRICE V., GILLIS S., HENNEY C.S., KRONHEIM S.R., GRABSTEIN K., CONLON P.J., HOPP, T.P. & COSMAN D. (1985) Cloning, sequence, and expression of two distinct human interleukin-1 cDNAs. *Nature (Lond.)*, **315**, 641.

MCADAM K.P.W.J., LI J., KNOWLES J., FOSS N.T., DINARELLO C.A., ROSENWASSER L.J., SELINGER M.J., KAPLAN M.M. & GOODMAN R. (1982) The biology of SAA: identification of the inducer, *in vitro* synthesis, and heterogeneity demonstrated with monoclonal antibodies. *Ann. N.Y. Acad. Sci.* **389**, 126.

NORTHEMANN W., ANDUS T., GROSS V., NAGASHIMA M., SCHREIBER G. & HEINRICH P.C. (1983) Messenger RNA activities of four acute phase proteins during inflammation. *FEBS Lett.* **61**, 319.

PEPYS M.B. & BALTZ M. (1983) Acute phase proteins. *Adv. Immunol.* **34**, 141.

RAMADORI G., SIPE J.D., DINARELLO C.A., MIZEL S.B. & COLTEN H.R. Pretranslational modulation of acute phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. *J. exp. Med.* **162**, 930.

RICCA G.A., HAMILTON R.W., MCLEAN J.W., CONN A., KALINYAK J.E. & TAYLOR J.M. (1981) Rat alpha 1-acid glycoprotein mRNA. Cloning of double-stranded cDNA and kinetics of induction of mRNA levels following acute inflammation. *J. biol. Chem.* **256**, 10362.

RITCHIE D.G. & FULLER G.M. (1983) Hepatocyte-stimulating factor: a monocyte-derived acute-phase regulatory protein. *Ann. N.Y. Acad. Sci.* **408**, 490.

SAKLATVALA J., PILSWORTH L.M.C., SARSFIELD S.J., GAVRILOVIC J. & HEATH J.K. (1984) Pig catobolin is a form of interleukin 1. *Biochem. J.* **224**, 461.

SCHREIBER G., HOWLETT G., NAGASHIMIA M., MILLERSHIP A., MARTIN H., URBAN J. & KOTLER L. (1982) The acute phase response of plasma response of plasma protein synthesis during experimental inflammation. *J. biol. Chem.* **257**, 10271.

SELINGER M.J., MCADAM K.P.W.J., KAPLAN M.M., SIPE J.D., VOGEL S.N. & ROSENSTREICH D.L. (1980) Monokine-induced synthesis of serum amyloid A protein by hepatocytes. *Nature (Lond.)*, **285**, 498.

SIPE J.D., VOGEL S.N., RYAN J.L., MCADAM K.P.W.J. & ROSENSTREICH D.L. (1979) Detection of a mediator derived from endotoxin-stimulated macrophages that induce the acute phase SAA response in mice. *J. exp. Med.* **150**, 597.

SIPE J.D., VOGEL S.N., SZTEIN M.B., SKINNER M. & COHEN A.S. (1982) The role of interleukin 1 in acute phase serum amyloid A (SAA) and serum amyloid P (SAP) biosynthesis. *Ann. N.Y. Acad. Sci.* **389**, 137.

VAN DAMME J., DE LEY M., OPDENAKKER G., BILLIAU A. & DE SOMER P. (1985) Homogeneous interferon-inducing 22K factor is related to endogenous pyrogen and interleukin-1. Nature (*Lond.*), **314**, 266.

WOLOSKI B.M.R.N.J. & FULLER G.M. (1985) Identification and partial characterization of hepatocyte-stimulating factor from leukemia cell lines: comparison with interleukin 1. *Proc. natl. Acad. Sci. U.S.A.* **82**, 1443.