Clin. exp. Immunol. (1987) **67**, 335–342

Decreased interleukin 1 activity in culture supernatant of lipopolysaccharide stimulated monocytes from patients with liver cirrhosis and hepatocellular carcinoma

M. YOKOTA, S. SAKAMOTO, S. KOGA & H. IBAYASHI *The Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan*

(Accepted for correspondence 30 July 1986)

SUMMARY

Immunoregulatory function of peripheral blood monocytes was studied in patients with hepatocellular carcinoma (HCC) and liver cirrhosis (LC), by assaying interleukin 1 (IL-1) and prostaglandin E_2 (PGE_2) in the culture supernatant of lipopolysaccharide-stimulated monocytes. IL-1 activity of the monocyte culture supernatant without indomethacin was decreased in patients with HCC and LC, compared with that of controls. The activity was lower in patients with HCC than that in those with LC. The PGE_2 content of the culture supernatant of monocytes from patients with LC and HCC was increased, compared to normal controls. To avoid the effect of PGE_2 on the IL-1 assay, we cultured the monocytes with addition of indomethacin and assayed IL-1 activity in the culture supernatant. As a result, monocyte IL-1 production was increased in patients with HCC and LC, compared with normal controls. The decrease in IL-1 activity of the supernatant without indomethacin of patients with LC and HCC was considered to be due to increased secretion of PGE_2 by the monocytes. Therefore, monocytes from patients with HCC and LC had an increased capacity of secreting both IL-1 and PGE_2 over normal controls, but the effect of the suppressor function (PGE_2 secretion) dominated in these patients.

Keywords interleukin 1 prostaglandin E_2 monocyte liver cirrhosis hepatocellular carcinoma

INTRODUCTION

Macrophage-monocyte lineage cell has an important regulatory role in immunological responses and inflammatory reaction (Unanue, 1981; Dinarello, 1984). This function of the cells seems to be mediated chiefly by two products of the monocytes, interleukin 1 (IL-1) (Gery & Waksman, 1972) and prostaglandin E_2 (PGE_2) (Bonney, 1978). IL-1 has a helper activity for lymphocyte activation and PGE_2 shows a suppressor activity to lymphocytes. We reported an increased secretion of IL-1 from monocytes in patients with chronic liver disease. The functions of suppressor monocytes are also augmented, possibly because of an increased secretion of PGE_2 in patients with chronic active hepatitis and liver cirrhosis (LC) (Sakamoto, Koga & Ibayashi, 1984) As hepatocellular carcinoma (HCC) develops frequently in patients with LC (Edmondson & Peters, 1982), it is considered that dysfunction of monocytes, especially the augmented function of suppressor monocytes may contribute to this tendency. We examined the immunoregulatory function of monocytes in patients with LC and HCC, by measuring IL-1 and PGE_2 in the culture supernatant of monocytes. We also evaluated the correlation between the IL-1 secretory potential of monocytes and body temperature

Correspondence: Dr M. Yokota, The Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Higashi-ku, Fukuoka 812, Japan.

or C-reactive protein (CRP) test, because it was reported that IL-1 was identical to endogenous pyrogen (Murphy, Simon & Willoughby, 1980; Rosenwasser, Dinarello & Rosenthal, 1979) and it enhances secretion of acute phase reactant from the liver (Merriman, Pulliam & Kampschmidt, 1975).

MATERIALS AND METHODS

Subjects. The patients studied were admitted to Kyushu University Hospital and related hospitals. HCC was diagnosed with angiography, aspiration cytology or autopsy. LC was diagnosed with liver biopsy, laparoscopy or clinical data. The patients were all Japanese and were divided randomly into two groups.

Group 1 patients for protocol 1. Eleven patients with LC (eight men and three women) and 13 patients with HCC (nine men and four women) were studied together with 10 healthy controls (six men and four women). Of LC patients, three were positive for HBsAg and three were alcoholics. Three HCC patients were positive for HBsAg. All HCC patients had complications of LC. The age range of the patients with LC and HCC and of controls was 35–64 years (mean 54 years), 53–79 years (mean 63 years) and 25–83 years (mean 51 years), respectively.

Group 2 patients for protocol 2. Sixteen patients with LC (11 men and five women) and 16 patients with HCC (12 men and four women) were studied. The controls included 11 men and five women. Five of the LC patients were HBsAg positive and three were alcoholics. Two of HCC patients were HBsAg positive. All HCC patients had complications of LC. The age range of the patients with LC and HCC and the controls was 43–63 years (mean 54 years), 47–77 years (mean 62 years) and 25–83 years (mean 45 years) respectively.

Laboratory data in both groups were compatible (Table 1). There was no significant difference between laboratory data of the patients with LC and HCC in group 1, but the activity of choline esterase of HCC (163 ± 56 iU/l) was significantly lower than that of LC (250 ± 103 iU/l) in the group 2 patients ($P < 0{\cdot}02$).

Table 1. Demographic and biochemical characteristics of the patients with liver cirrhosis (LC) and hepatocellular carcinoma (HCC)

	Group 1		Group 2	
Diagnosis	LC	HCC	LC	HCC
No. patients	11	13	16	16
Age (years)	54 ± 9	63 ± 7	54 ± 7	62 ± 8
Sex				
M	8	9	11	12
F	3	4	5	4
Albumin (g/dl)	$3{\cdot}6 \pm 0{\cdot}8$	$3{\cdot}4 \pm 0{\cdot}5$	$3{\cdot}6 \pm 0{\cdot}9$	$3{\cdot}4 \pm 0{\cdot}6$
γ-Globulin (g/dl)	$2{\cdot}1 \pm 0{\cdot}7$	$2{\cdot}3 \pm 0{\cdot}7$	$2{\cdot}0 \pm 0{\cdot}6$	$2{\cdot}3 \pm 0{\cdot}7$
AST (iu/l)	102 ± 58	113 ± 54	106 ± 58	106 ± 49
Choline esterase (iu/l)	221 ± 79	164 ± 63	250 ± 103	163 ± 56
			—$P < 0{\cdot}02$—	
ICG_{R15}	34 ± 16	32 ± 12	31 ± 18	35 ± 15

AST, Aspartate aminotransferase.

ICG_{R15} Indocyanine green retention rate at at 15 min.

Laboratory data in both groups were compatible. There was no significant difference between laboratory data of the patients with LC and HCC in group 1, but the activity of choline esterase of HCC was significantly lower than that of LC in the group 2 patients.

Preparation of lipopolysaccharide stimulated monocyte culture supernatant. The method used was as described (Sakamoto *et al.*, 1984) Briefly, mononuclear cells were obtained from peripheral blood centrifugation at 1550 rev/min for 45 min over Ficoll-Conray. The mononuclear cells were suspended in 5 ml of RPMI 1640 (Nissui Seiyaku, Japan) containing 10% (V/V) of heat-inactiviated fetal calf serum (FCS, GIBCO, Grand Island, NY). RPMI 1640 was supplemented with 20 mM Hepes and 1 μg/ml of gentamycin. The cells were cultured in a 5 ml plastic dish (Falcon 3002, Becton, Dickinson and Co., Cockeysville, MD) for 1 h in an atmosphere of 5% CO_2. Over 95% of the adherent cells were monocytes, as determined by nonspecific esterase staining, and confirmed morphologically. The adherent cells served as the monocyte preparation.

The monocytes were incubated using two different incubation systems (protocol 1 and 2) and the culture supernatants were harvested. In protocol 1, 1×10^6 monocytes were suspended in 1 ml of the supplemented RPMI 1640 containing 10% FCS and 20 μg/ml of lipopolysaccharide of *Salmonella typhimurium* (LPS, Difco, Detroit, MI) and incubated at 37°C under an atmosphere of 5% CO_2 for 24 h. In protocol 2, 5×10^5 monocytes were cultured with 20 μg/ml of lipopolysaccharide and 1 μg/ml of indomethacin (Sumitomo Seiyaku, Japan, dissolved in ethanol) to block release of prostaglandin E_2 or to suppress the function of suppressor cells.

After the incubation, the culture medium was centrifuged at 1100 rev/min for 10 min and the supernatant was used as the LPS stimulated monocyte culture supernatant. We cultured the monocytes from one healthy 29-year-old man as a reference at the time of each culture, and the IL-1 activity and PGE_2 concentration of this culture supernatant served as the standards of IL-1 activity and PGE_2 concentration.

Culture of thymocyte for IL-1 assay. Thymuses were aseptically removed from C3H/HeJ mice obtained from Jackson Laboratory (Bar Harbor, ME). A single cell suspension of thymocytes was prepared by gently pressing the organs through a 200 mesh stainless steel net. Thymocytes, 1×10^6, in 0·2 ml of RPMI 1640 containing 5% FCS were cultured with 5·0 μg/ml of phytohemagglutinin-(PHA, Difco, Detroit, MI) in a Falcon 3072 plate for 72 h at 37°C. The cultures were pulsed with 0·2 μCi of tritiated thymidine (^{3}H-TdR, 5 Ci/mmol, RCC Amersham, England) for the final 20 h. The cells were collected on a glass fibre filter by a Mash II cell harvester (Microbiological Association, Rockville, MD), and the filters were washed with distilled water and counted for radioactivity in a scintillation counter.

Assay of IL-1 activity. This assay was done in triplicate. LPS stimulated monocyte culture supernatant was added to the thymocyte culture (25%, v/v) described above. The difference in the uptake of ^{3}H-TdR in the presence and absence of the monocyte culture supernatant was defined as IL-1 activity. As described (Sakamoto *et al.*, 1984), IL-1 activities in each sample were expressed in percent, compared wih the IL-1 activity obtained from the reference person, determined in each experiment. The formula used for calculation is as follows.

$$\text{IL-1 activity} = \frac{\Delta^3\text{H-TdR uptake of the thymocyte cultured with patient's supernatant}}{\Delta^3\text{H-TdR uptake of the thymocyte cultured with reference supernatant}} \times 100\ (\%)$$

The difference in the uptake of ^{3}H-TdR in the presence and absence of the monocyte culture supernatant was defined as Δ^3H-TdR uptake.

IL-1 act-ind⊖ represents IL-1 activity of LPS-stimulated monocyte culture supernatant, without indomethacin.

IL-1 act-ind⊕ represents IL-1 activity of LPS-stimulated monocyte culture supernatant, with indomethacin. As this activity was not decreased by treatment of trypsin (Sakamoto *et al.*, 1984), this enhancing activity on thymocyte proliferation should be that of IL-1 since IL-1 was reported to be resistant to the treatment of trypsin (Wood, 1979).

Assay of PGE_2. PGE_2 contents in the culture supernatant were assayed using a RIA Kit (Prostaglandin E_2 [^{125}I] RIA Kit, New England Nuclear, Boston, MA). PGE_2 concentration was expressed as a % of PGE_2 concentration of the monocyte culture supernatant from the reference person, included in each experiment. The formula used for calculation is as follows.

$$PGE_2 = \frac{PGE_2 \text{ concentration of the patient's supernatant}}{PGE_2 \text{ concentration of the reference's supernatant}} \times 100\ (\%)$$

Body temperature of the patients. The body temperature was measured three times daily at 0600, 1000 and 1400 h. The highest body temperature of each patient during 3 days before the monocyte culture was defined as the body temperature in this study.

C-reactive protein (CRP) test. The CRP test was performed using the capillary tube technique (Anderson & McCarty, 1950). The results were expressed as negative (−), pseudo-positive (±) and positive (+–4+).

Statistical analysis. Statistical analysis of IL-1 activity and PGE_2 was performed using the Wilcoxon's rank sum test. Correlation between IL-1 act-ind⊕ and CRP was determined by Wilcoxon's rank sum test. Correlation between IL-1 act-ind⊕ and body temperature was determined by linear regression analysis, and the significance of variance from the observed regression coefficient was assessed using Student's *t*-test. Probability values below 5% were considered significant.

RESULTS

Interleukin 1 activity of LPS-stimulated monocyte culture supernatant without indomethacin (IL 1 act-ind⊖) (protocol 1). Figure 1 shows the IL 1 act-ind⊖ of Protocol 1. Mean IL-1 act-ind⊖ with standard deviation (s.d.) of 10 controls was 98·4 ± 13·4%. The values for patients with LC and HCC were 87·7 ± 9·3% and 78·1 ± 11·2%, respectively, and were significantly lower than data of normal controls ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively). Moreover, IL-1 act-ind⊖ of patients with HCC was lower than that of LC ($P < 0{\cdot}05$).

PGE_2 concentration in the culture supernatant (protocol 1). Figure 2 illustrates the PGE_2 concentration of the culture supernatant of Protocol 1. Mean PGE_2 concentration with standard deviation of 10 controls was 98·2 ± 47·7%. The values for patients with LC and HCC were 165·3 ± 70·4% and 174·9 ± 73·0%, respectively. The values were significantly higher than data from normal controls ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively).

Interleukin 1 activity of LPS-stimulated monocyte culture supernatant with indomethacin (IL-1 act-ind⊕) (protocol 2). To exclude the effect of prostaglandin on the IL-1 assay, indomethacin was added to the culture of monocytes to inhibit prostaglandin synthesis. IL-1 act-ind⊕ of the patients

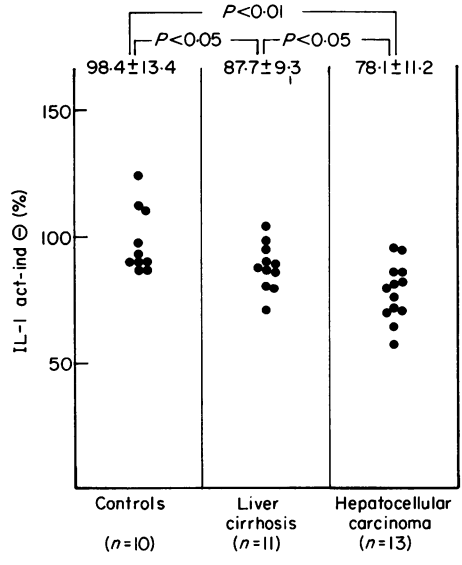

Fig. 1. Interleukin 1 activity of LPS-stimulated monocyte culture supernatant without indomethacin (IL-1 act-ind⊖).

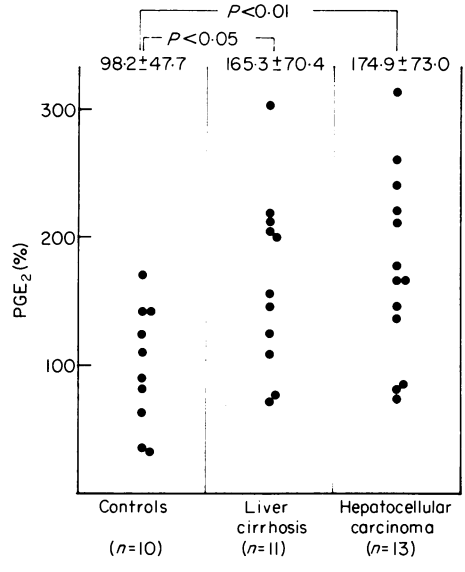

Fig. 2. Prostaglandin E_2 of LPS-stimulated monocyte culture supernatant without indomethacin.

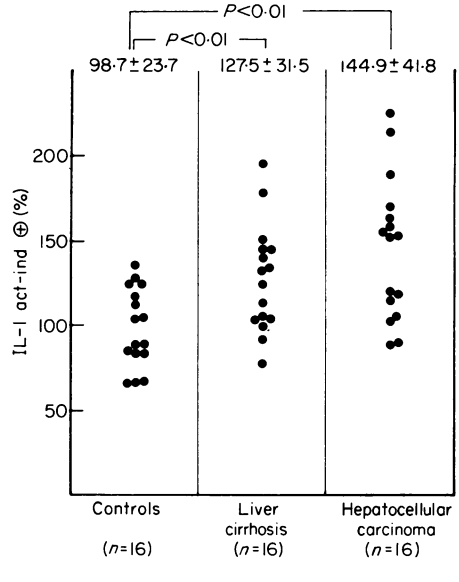

Fig. 3. Interleukin 1 activity of LPS-stimulated monocyte culture supernatant with indomethacin (IL-1 act-ind⊕).

with LC and HCC showed an increase when compared to that of normal controls ($P<0·01$, $P<0·01$) (Fig. 3). IL-1 act-ind⊕ of the control, LC and HCC was 98·7±23·7%, 127·5±31·5% and 144·9±41·8%, respectively.

Correlation between CRP, body temperature and IL-1 act-ind⊕. As IL-1 was considered to be identical to endogenous pyrogen (Murphy *et al.*, 1980; Rosenwasser *et al.*, 1979), and it enhances secretion of acute phase reactant from the liver (Merriman *et al.*, 1975), we examined whether the IL-1 activities were related to these biological responses in our patients. In LC, IL-1 act-ind⊕ had a

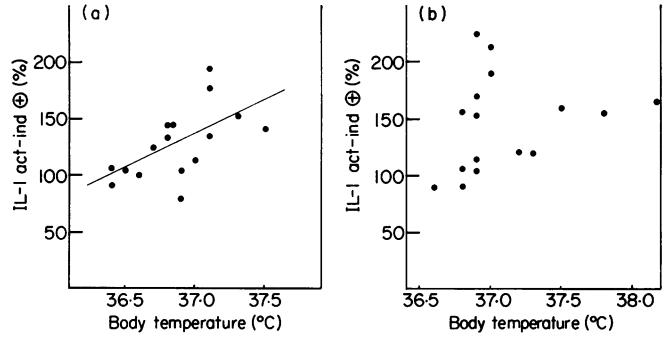

Fig. 4. Correlation between IL-1 act-ind⊕ and body temperature in (a) liver cirrhosis and (b) hepatocellular carcinoma. Body temperature was defined as highest body temperature of each patient during 3 days before the monocyte culture. (a) $r=0{\cdot}56$, $n=16$, $P<0{\cdot}05$; $r=0{\cdot}175$, not significant.

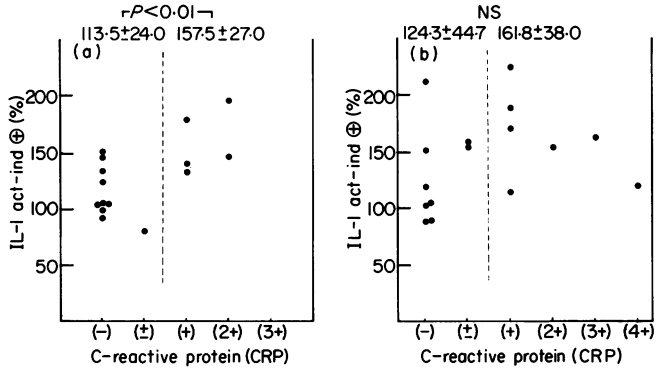

Fig. 5. Correlation between IL-1 act-ind⊕ and CRP in (a) liver cirrhosis and (b) hepatocellular carcinoma. NS, not significant.

positive correlation with the body temperature ($r=0{\cdot}56$, $P<0{\cdot}05$) (Fig. 4a), and IL-1 act-ind⊕ of CRP positive patients was higher than that of the CRP negative or pseudo-positive patients ($P<0{\cdot}01$) (Fig. 5a). In HCC however, the body temperature and the CRP test did not correlate with IL-1 act-ind⊕, thereby suggesting additional factors which affect body temperature and CRP production (Fig. 4b) (Fig. 5b).

DISCUSSION

In this study, we found a decreased IL-1 act-ind⊖ in patients with LC and HCC, as compared with normal controls. This suggests a decrease in the activity of helper function of monocytes in these patients. We also found a lower IL-1 act-ind⊖ in patients with HCC, thereby suggesting that the helper function of monocytes in HCC was depressed to a greater extent than was seen in LC.

In a previous study, we showed that decreased IL-1 act-ind⊖ seen in the patients with chronic liver disease was related to the degree of liver dysfunction (Sakamoto *et al.*, 1984). However, the lower activity of IL-1 act-ind⊖ of the HCC patients observed in this study was not simply due to the differences of the liver function between these patients, since the survey of liver function of HCC and LC patients of the protocol 1 showed no difference. Factors other than liver dysfunction should be considered to relate to the decrease of IL-1 act-ind⊖ in HCC patients. As it was reported that IL-1 activity in the monocyte culture supernatant was decreased in patients with cancer (Finelt &

Hoffmann, 1979), the development of HCC itself might decrease the activity of IL-1 in the monocyte culture supernatant of patients with HCC, or LC patients with a lower IL-1 act-ind⊖ may be more prone to develop HCC.

There are at least two mechanisms related to the decrease of IL-1 activity in a monocyte culture supernatant without indomethacin. (1) Decreased secretion of IL-1 from monocytes, and (2) increased secretion of inhibitory factors of IL-1 activity, especially PGE_2 (increased activity of suppressor monocyte). Although the IL-1 act-ind⊖ was decreased in patients with LC and HCC (Fig. 1), the IL-1 act-ind⊕ was increased compared with normal controls, thereby indicating increased secretions of IL-1 in these patients (Fig. 3). The assay of PGE_2 showed an increased secretion of PGE_2 in patients with LC and HCC (Fig. 2). Thus, this increase in PGE_2 secretion was a major factor involved in the decrease of IL-1 activity in the monocyte culture supernatant.

There was no difference in PGE_2 secretory potentials between the patients with HCC and LC, and also no difference of IL-1 act-ind⊕ between these patients, although IL-1 act-ind⊖ did differ (Fig. 1).

It was reported that IL-1 facilitated the secretion of the PGE_2 (Dinarello, Marnoy & Rosenwasser, 1983). The increased secretion of the IL-1 (IL-1 act-ind⊕) in patients LC and HCC shown in this study may facilitate PGE_2 secretion from monocytes. In other words, excessive 'helper signal' as a result of increased IL-1 secretion may augment the function of suppressor monocytes.

It has been reported that PGE_2 decreases natural killer (NK) cell activity (Brunda, Herberman & Holden, 1980), and NK cell activity is depressed in patients with LC (Nakamura *et al.*, 1983). Because NK cells play an important role in the control of tumor growth (Haller *et al.*, 1977; Talmadge *et al.*, 1980), this excessive production of PGE_2 from monocytes might contribute to development of HCC in LC.

While the mechanism of increased production of IL-1 and PGE_2 in monocytes of patients with LC and HCC was not clarified, the following possibility should be considered. A subset or subsets of monocytes with a high reactivity to exogenous stimuli might be increased, or monocytes may be stimulated by certain increased serum factors in patients with liver dysfunctions. Serum factors which affect the function of monocyte have been noted in patients with LC (Hassner *et al.*, 1981; Holdstock, Chastenay & Krawitt, 1982).

IL-1 is considered to be identical to endogenous pyrogen (Murphy *et al.*, 1980; Rosenwasser *et al.*, 1979) and it enhances secretion of acute phase reactant from the liver (Merriman *et al.*, 1975). In this study, we demonstrated the relationship between body temperature or CRP and the productivity of IL-1 of monocytes from LC patients. These results suggest that our monocyte function test *in vitro* (determination of IL-1 and PGE_2 in the monocyte culture supernatant) represents monocyte function *in vivo*. However, as the relation was not evident in the HCC patients, additional factors probably affect body temperature and CRP production in HCC patients. In fact, interferon α and cachectin were reported to have pyrogenic activity recently (Dinarello *et al.*, 1984; 1986). Further studies will be necessary to know how these factors contribute to the regulation of body temperature.

We thank Dr K. Hiroshige and Dr T. Hirata for the kind provision of blood samples from patients under their care.

REFERENCES

ANDERSON, H.C. & MCCARTY, M. (1950) Determination of C-reactive protein in blood as measure of activity of disease process in acute rheumatic fever. *Am. J. Med.* **8,** 445.

BONNEY, R.J., WIGHTMAN, P.D., DAVIES, P., SADOWSKI, S.J., KUEHL, F.A. & HUMES, J.L. (1978) Regulation of prostaglandin synthesis and of the selective release of lysosomal hydrolases by mouse peritoneal macrophages. *Biochem. J.* **176,** 433.

BRUNDA, M.J., HERBERMAN, R.B. & HOLDEN, H.T. (1980) Inhibition of murine natural killer cell activity by prostaglandins. *J. Immunol.* **124,** 2682.

DINARELLO, C.A. (1984) Interleukin-1 and the pathogenesis of the acute-phase response. *N. Engl. J. Med.* **311,** 1413.

DINARELLO, C.A., BERNHEIM, H.A., DUFF, G.W., LE, H.V., NAGABHUSHAN, T.L., HAMILTON, H.C. & COCEANI, F. (1984) Mechanisms of fever induced by recombinant human interferon. *J. clin. Invest.* **74,** 906.

DINARELLO, C.A. CANNON, J.G., WOLFF, S.M. BERNHEIM, H.A., BEUTLER, B., CERAMI, A., FIGARI, I.S., PALLADINO, M.A. JR. & O'CONNOR, J.V. (1986) Tumor necrosis factor (cachectin) is an endogenous

pyrogen and induces production of interleukin 1. *J. exp. Med.* **163,** 1433.

DINARELLO, C.A., MARNOY, S.O. & ROSSENWASSER, L.J. (1983) Role of arachidonate metabolism in the immunoregulatory function of human leukocytic pyrogen lymphocyte activating factor Interleukin 1. *J. Immunol.* **130,** 890.

EDMONDSON, H.A. & PETERS, R.L. (1982) Neoplasmas of the liver. In *Disease of the Liver* (ed. by L. Shiff & E.X. Shiff) p. 1101. J.B. Lippincott, Philadelphia.

FINELT, M. & HOFFMANN, M.K. (1979) A human monocyte function test: release of B-cell differentiation factor. *Clin. Immunol. Immunopathol.* **12,** 281.

GERY, I. & WAKSMAN, B.H. (1972) Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediator(s). *J. exp. Med.* **136,** 143.

HALLER, O., HANSSON, M., KIESSLING, R. & WIGZELL, H. (1977) Role of non-conventional natural killer cells in resistance against syngeneic tumor cells in vivo. *Nature* **270,** 609.

HASSNER, A., KLETTER, Y., SHLAG, D., YEDVAB, M., ARONSON, M. & SHIBBOLET, S. (1981) Impaired monocyte function in liver cirrhosis. *Brit. med. J.* **282,** 1262.

HOLDSTOCK, G., CHASTENAY, B.F. & KRAWITT, E.L. (1982) Studies on lymphocyte hyporesponsiveness in liver cirrhosis; the role of monocyte suppressor cell activity. *Gastroenterology* **82,** 206.

MERRIMAN, C.R., PULLIAM, L.A. & KAMPSCHMIDT, R.F. (1975) Effect of leukocytic endogenous mediator on C-reactive protein in rabbits. *Proc. Soc. exp. Biol. Med.* **149,** 782.

MURPHY, P.A., SIMON, P.L. & WILLOUGHBY, W.F. (1980) Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte activating factors made by rabbit alveolar macrophages. *J. Immunol.* **124,** 2498.

NAKAMURA, T., MORIZANE, T., WATANABE, T., TSUCHIMOTO, K., INAGAKI, Y., KUMAGAI, N. & TSUCHIYA, M. (1983) Decreased natural killer activity in patients with liver cirrhosis. *Int. J. Cancer* **32,** 573.

ROSENWASSER, L. J., DINARELLO, C.A. & ROSENTHAL, A.S. (1979) Adherent cell function in murine T lymphocyte antigen recognition. Enhancement of murine T cell antigen recognition by human leukocytic pyrogen. *J. exp. Med.* **150,** 709.

SAKAMOTO, S., KOGA, S. & IBAYASHI, H. (1984) Interleukin 1 activity in culture supernatant of lipopolysaccharide-stimulated monocytes from patients with chronic liver disease. *Hepatogastroenterol.* **31,** 248.

TALMADGE, J.E., MEYERS, K.M., PRIEUR, D.J. & STARKEY, J.R. (1980) Role of NK cells in tumor growth and metastasis in beige mice. *Nature* **284,** 622.

UNANUE, E.R. (1981) The regulatory role of macrophages in an antigen stimulation between lymphocyte and macrophage. In: *Advances in Immunology.* Vol. 31 (ed. by H.G. Kunkel & J.F. Dexon, p. 1. Academic Press, New York.

WOOD, D.D. (1979) Purification and properties of human B cell activating factors. *J. Immunol.* **123,** 2395.