

## Monocyte function in cirrhosis

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**SUMMARY** Monocyte function has been studied in a total of 51 patients with biopsy-proven cirrhosis and 35 controls. There was significantly reduced monocyte spreading ( $p < 0.05$ ), chemotaxis ( $p < 0.02$ ), bacterial phagocytosis ( $p < 0.05$ ) and bacterial killing ( $p < 0.02$ ) in the cirrhotics compared to the controls. Monocytes from patients with cirrhosis produced significantly less of the lysosomal enzymes N-acetyl  $\beta$ -glucosaminidase and  $\beta$ -glucuronidase than those obtained from the controls ( $p < 0.02$ ). There was no significant difference in the number of monocytes obtained, the number of macrophage precursors, and the nitro-blue tetrazoline (NBT) reduction between the cirrhotic and the controls. The reduced function appeared to be mainly due to a circulating inhibitory factor and could be corrected by incubation of the cirrhotic cells in serum from control subjects. The response of monocytes from patients with cirrhosis did not differ from the controls in their response to added endotoxin or latex particles suggesting that they are capable of a normal response in the absence of the inhibitory factor. Paired specimens of portal and systemic serum were collected from patients with no evidence of liver disease undergoing vascular surgery. When added to normal human monocytes the portal serum caused a significant reduction in bacterial killing ( $p < 0.02$ ) and chemotaxis ( $p < 0.05$ ) compared to results obtained in the paired systemic serum. Mixing experiments suggests the presence of an active inhibitor in the portal serum.

The results suggest that monocyte function is reduced in cirrhosis apparently due to a serum inhibitor which may have originated from the portal vein. The abnormalities may account in part for the increased susceptibility of these patients to infection.

A variety of defects in the cellular immune system have been described in liver disease, but the literature concerning monocyte function in cirrhosis is scarce. The predisposition of patients with alcoholism and cirrhosis to infection<sup>1-3</sup> is well recognised, and alcoholics may show varying degrees of leucopenia<sup>4</sup> and leucocyte functional derangement.<sup>5</sup> Von Epps and coworkers<sup>6</sup> showed that inhibitors to both leucocyte and monocyte chemotaxis was present in 50% of patients with alcoholic liver disease and that the severity of the defect correlated with serum IgG and particularly IgA concentrations. DeMeo<sup>7</sup> also showed chemotactic inhibitory substance activity in serum from 19 of 22 patients with alcoholic liver disease, at least three different chemotactic inhibitors being present. Hassner<sup>8</sup> demonstrated reduced phagocytosis and killing of *Candida albicans* in patients with cirrhosis and suggested the presence of a serum inhibitory factor. On the contrary Ganguly *et al*<sup>9</sup> suggested that the

production of acid hydrolase enzymes from monocytes was increased in cirrhotics, suggesting that the cells were "activated". These studies of monocyte function in cirrhosis are of interest not only because they may account for the increased susceptibility of patients to infection but also because they may be an indicator of Kupffer cell function.<sup>10</sup>

It has been postulated that, in cirrhosis, antigens absorbed from the gastrointestinal tract may not be removed by the failing liver either through shunting of portal blood, or because of impairment of Kupffer cell function. These antigens may then result in continuous immunostimulation leading to immunosaturation and a reduced response to further superimposed infection.<sup>11 12</sup> These antigens could be similar to the inhibitory substance discussed above.

The purpose of this paper is to try to define more accurately monocyte defects in patients with cirrhosis and to investigate the possibility of an over-spill phenomenon contributing to the abnormalities found in cirrhosis.

## Patients and methods

### PATIENTS

Only patients with biopsy-proven liver disease were studied. Where indicated below, and where numbers permitted, the patients were classified as "active" or "quiescent" on the basis of the biopsy, biochemical, and clinical assessment. The former were mainly in-patients, the latter out-patients. Age and sex-matched controls were selected from hospital staff and from patients attending a day-ward for minor surgery. A standard full blood count and differential white count was performed. Eleven alcoholic patients with no evidence of hepatitis or cirrhosis on biopsy and six patients with obstructive jaundice were also studied. All studies were approved by the district ethical committee.

### CULTURE CONDITIONS

A mixed mononuclear cell preparation (MMC) containing approximately 30% of monocytes was obtained from fresh venous heparinised (10 U/ml) blood by density centrifugation following layering on to lymphocyte preparation medium (Flow Laboratories, UK). This was then washed three times in Hanks' buffered salt solution (HBSS). A monocyte-enriched preparation (MEC) was obtained by adherence to plastic Petri dishes for two hours at which time nonadherent cells were removed. The adherent cells were then washed and harvested using a rubber policeman and then washed again. Viability was confirmed by exclusion of trypan blue. Cytospin preparations were stained with either Giemsa-Wright or non-specific esterase and the MEC preparation was confirmed to contain approximately 90% monocytes. Any preparation with a viability of less than 90% or which contained less than 80% of monocytes was discarded.

### TESTS OF MONOCYTE FUNCTION

#### *Macrophage precursors*

The macrophage precursor assay described by Currie and Hedley<sup>13</sup> was used. In brief, MMC ( $2 \times 10^6$ /ml) in RPMI 1640 (+25 mmol HEPES and antibiotics) containing 50% autologous serum was added in 100  $\mu$ l volumes to the wells of a plastic microplate. Plates were incubated at 37° in 5% CO<sub>2</sub> in humid air for seven days, when the wells were washed free of unattached cells with HBSS and 50  $\mu$ l of a 0.01 M citric acid solution containing 1:2000 crystal violet added to each well.<sup>14</sup> The plates were allowed to stand for 30 min at room temperature at which time the detached nuclei were counted in a haemocytometer. This assay is thought to indicate the number of circulating monocytes present which

have the capacity to mature into tissue macrophages.

#### *Monocyte spreading*

Spreading was measured using the method described by Territo and Cline.<sup>15</sup> In brief, 0.5 ml of a  $4 \times 10^6$  cell/ml MMC preparation was placed over glass coverslips in sterile plastic multiplates (Lux Scientific Corporation). After 1, 24, and 48 h coverslips were removed, gently washed in HBSS and fixed in 2% buffered glutaraldehyde for 5 min. The coverslips were then inverted on to oil drops in glass slides, taking care to avoid drying. The longest axis of the cells was measured using phase-contrast microscopy with an oil-immersion objective lens (each reading mean of 20). This ability to spread is increased when the monocytes are "activated" and is probably a measure of cell maturity.

### NITRO-BLUE TETRAZOLIUM REDUCTION

The redox dye nitro-blue tetrazolium (NBT) is reduced by the action of NADPH oxidase. The product is the insoluble coloured crystalline formazan. The assay is therefore an indirect measurement of the hexose monophosphate shunt. The method described by Hedley and Curry,<sup>16</sup> itself an adaptation<sup>17</sup> was employed. In brief, 150  $\mu$ l of a MMC suspension containing  $10^6$  mononuclear cells was placed in 2 ml polypropylene tubes. As a phagocytic stimulus, 50  $\mu$ l of 0.79  $\mu$ m latex polystyrene beads (Sigma) diluted in 1/100 in RPMI 1640 was added and 50  $\mu$ l of RPMI 1640 added to control tubes. After incubation at 37°C for 15 min, 25 ml of a 4 nmol solution of NBT (Sigma) in 314 nm sucrose were added. After a further one hour incubation, the reaction was stopped by adding one drop of 0.1 N HCl to the tubes. The formazan was then extracted by adding 500  $\mu$ l of Dioxan to the cell button and incubation at 70°C for 20 min. After centrifugation at 2000 g for 15 min, the clear supernatant was read at 520 nm on a spectrofluorometer, using Dioxan as a blank. A standard curve was prepared by reducing doubling dilutions of NBT with 150 nmol of ascorbic acid.<sup>18</sup>

### PHAGOCYTOSIS AND BACTERIAL KILLING OF STAPHYLOCOCCUS AUREUS

The method used was based on that described by Territo and Cline<sup>15</sup> with minor modifications.<sup>19,20</sup> The method below produced the most reproducible results in preliminary studies. *Staph aureus* was cultured overnight in a brain/heart medium and diluted to a final concentration of approximately  $2 \times 10^6$  bacterial colony-forming units (CFU) per ml. This was divided into aliquots, snap-frozen and stored at -70°C for later use. For phagocytosis, 0.125  $\mu$ l of a

MMC preparation in a concentration of  $4 \times 10^6$  cells was added to 0.125  $\mu$ l of autologous serum and 0.125  $\mu$ l of bacterial suspension in a 12  $\times$  75 mm plastic tube. After incubation at 37°C for one hour, a cytospin preparation was prepared and the number of bacteria ingested by 100 monocytes counted (mean of five readings). To measure bacterial killing 200  $\mu$ l of bacterial solution was put in a 12  $\times$  75 mm plastic tube. To this was added 200  $\mu$ l autologous serum and 500  $\mu$ l of a mixed mononuclear cell preparation containing  $4 \times 10^6$  cells/ml. This was incubated for one hour at 37°C, sonicated to disrupt cells and release bacteria (confirmed microscopically) and diluted 1/1000. The number of CFU/ml was then measured by culturing on blood-agar plates for 24 h. The percentage killing was calculated from the equation: % killing =  $A - B/A$ , where A is CFU/ml for the control cultures—that is, tube containing only bacteria and no cells, and B is CFU/ml count for tube containing bacteria and cells.

#### CHEMOTAXIS

Chemotaxis assays were performed by a modification of the Boyden chamber technique<sup>21</sup> as described by Wilkinson.<sup>22</sup> Serum chemotactic activity was produced by incubating fresh frozen human AB serum with zymosan (Sigma Chemical Co 0.5 mg/ml serum). A mixed mononuclear cell preparation of  $1 \times 10^6$  cells in 0.5 ml was added to the top chamber with no added serum. Distance moved by monocytes through an 8  $\mu$ m millipore filter was measured after incubation at 37°C for 4 h in a CO<sub>2</sub> incubator using the leading front counting technique as described by Zigmond and Hirsch.<sup>23</sup> The mean of 10 readings was recorded. An assessment of random movement was obtained by the measurement of migration of mononuclear cells exposed to RPMI 1640 alone (containing no zymosan-treated serum). This measurement was subtracted from the movement induced by the zymosan-treated serum to obtain the measurement of chemotaxis.

#### ACID HYDROLASE PRODUCTION

Production of the acid hydrolase N-acetylglucosaminidase (NAG) and  $\beta$ -glucuronidase ( $\beta$ G) was measured using the methods described in detail elsewhere.<sup>9</sup> HEPES-buffered RPMI 1640 supplemented with 10% fetal calf serum (SRPMI 1640) was used for cell maintenance to each of six wells of a sterile plastic multiplate (Lux Scientific Corporation) monocytes enriched cells ( $1.5 \times 10^6$ ) in SRPMI 1640 were added and allowed to adhere to the surface by incubation at 37°C in a 5% carbon dioxide fully humidified atmosphere for two hours with occasional agitation. After incubation, supernatant was discarded and three of the wells were

filled with 1.5 ml of an endotoxin solution and three with S-RPMI 1640 alone. The plates were incubated for a further two hours and the supernatants discarded, the cells washed three times in HBSS to remove the endotoxin and refilled with 1.5 ml of S-RPMI 1640. Following a further 24-hour incubation, supernatants were collected from each well, centrifuged to remove any contaminating cells, and stored in plastic tubes at -20°C. Adherent cells were removed from the plastic surface of each well with a rubber policeman into 1.5 ml of sterile ice-cold distilled water and the cell suspension was rapidly frozen and thawed eight times to lyse the monocytes. It was not possible to carry out a cell count before lysis, and we have assumed that the cell number after incubation bore a constant relation to the starting inoculum in each case. The total protein concentration of cell lysate was estimated by the method of Lowry *et al*<sup>24</sup> and was used as an index of cell mass.<sup>25</sup> A sensitive assay for NAG and  $\beta$ G was developed from the spectrofluorometric method of Beutler *et al*<sup>25</sup> as described previously.<sup>9</sup> The endotoxin solution *Salmonella typhosa* 0901 (Difco Laboratories) was prepared in 0.85% sodium chloride at a concentration of 1 mg/ml and diluted with RPMI 1640 medium to give a working concentration of 50  $\mu$ g/ml.

#### FURTHER EXPERIMENTS

Monocytes from a normal human volunteer were incubated at 37°C for four hours in paired specimens of serum (20%) from the controls and from cirrhotics. The cells were then washed three times prior to measurement of chemotaxis and bacterial killing. In these experiments the bacterial killing and phagocytosis was performed in fetal calf serum. In further experiments chemotaxis and bacterial killing were measured on cells from cirrhotic patients and incubated with control serum (conditions as above) and compared to the results obtained by those incubated in autologous serum. To investigate the overspill phenomenon, paired specimens of portal serum and venocaval serum were collected. These were obtained from patients undergoing vascular reconstructive surgery. None of these patients had clinical or biochemical evidence of liver disease. The effect of the paired serum specimens was measured on chemotaxis and bacterial killing properties of monocytes from healthy volunteer donors (methods as for studies comparing cirrhotic and control serum).

#### STATISTICS

Student's *t* test was used throughout. The Mann-Whitney U test was also used for comparing non-parametric data. Probability values of less than 5%

Table 1 Numbers of monocyte and glass adherent cells in patients and controls (mean  $\pm$  SEM)

	No of subjects	Absolute No of monocytes in blood film (no/ml)	No of glass adherent cells recovered from 50 ml blood $\times 10^6$
Controls	35	538 $\pm$ 42	7.0 $\pm$ 3.4
Chronic active hepatitis	10	594 $\pm$ 54	6.0 $\pm$ 4.5
Alcoholic cirrhosis	25	512 $\pm$ 48	6.6 $\pm$ 4.5
Alcoholic non-cirrhotic	11	594 $\pm$ 84	8.2 $\pm$ 5.01
All other cirrhotics	16	524 $\pm$ 34	5.37 $\pm$ 6.7
Obstructive jaundice	6	582 $\pm$ 39	7.9 $\pm$ 5.6
All cirrhotics	51	528 $\pm$ 56	6.1 $\pm$ 5.6

were considered to be significant. All assays were performed in triplicate and were coded and read without knowledge of the patients' clinical details. The results are expressed as mean  $\pm$  SEM. Inter-assay variability was less than 15% for all the assays.

## Results

Not all the tests of monocyte function were measured concurrently. Table 1 shows the total number of patients studied, the nature of the liver disease, and the total number of monocytes in the blood film and number of adherent cells recovered for each group. There was no significant difference between controls and cirrhotics. Although the number of macrophage precursors was slightly reduced in the cirrhotics ( $9.2 \pm 3.6 \times 10^4/\text{ml}$  for the controls, and  $8.8 \pm 4.2 \times 10^4/\text{ml}$  for the cirrhotics,  $n = 16$ ), the difference did not reach statistical significance. The results for monocyte spreading in controls ( $n = 15$ ) were  $12.4 \mu\text{m} \pm 2.4$ ,  $17.4 \mu\text{m} \pm 2.2$ , and  $19.4 \mu\text{m} \pm 3.4$  at time 0, 24, and 48 h respectively. Corresponding values for the cirrhotics ( $n = 15$ ) were  $12.8 \mu\text{m} \pm 2.7$ ,  $14.4 \mu\text{m} \pm 1.8$ , and  $16.8 \mu\text{m} \pm 3.4$ . The differences were small and only reached statistical significance at 24 h ( $p < 0.05$ ). The reduction of NBT was  $12.2 \pm 4.0 \times 10^{-15}$  per unstimulated monocyte, and  $14.2 \pm 3.6 \times 10^{-15}$  per monocyte stimulated with latex for the controls, and  $9.6 \pm 3.8 \times 10^{-15}$  and  $12.4 \pm 4.2 \times 10^{-15}$  per monocyte for the cirrhotics. Thus, NBT reduction tended to be lower in the cirrhotics, but again the difference did not reach statistical significance. Both controls and cirrhotics responded to the latex beads in a similar fashion and the increase expressed as a percentage was not significantly different in the two groups.

Results of phagocytosis, bacterial killing and chemotaxis are shown in Table 2. Both phagocytosis and chemotaxis were significantly reduced in the cirrhotics. Although reduced, the overall difference in killing between the cirrhotics and the controls did not reach statistical significance, but, comparing those with active disease ( $n = 10$ ) there was a significant reduction ( $29\% \pm 3.8$  v  $40\% \pm 3.6$ ,  $p < 0.02$ ), suggesting that the more severe the disease,

Table 2 Results of chemotaxis, bacterial phagocytosis and killing in cirrhotics and control patients (mean  $\pm$  SEM)

	Chemotaxis ( $\mu\text{m}$ ) ( $n = 24$ )	Phagocytosis No/100 cells ( $n = 16$ )	Killing (%) ( $n = 30$ )
Cirrhotic patients	12 $\pm$ 6 $p < 0.02$	123 $\pm$ 18.4	34 $\pm$ 4 NS
Controls	39.2 $\pm$ 3.8	149 $\pm$ 28.4	40 $\pm$ 3.6

the greater the loss of function. In the case of bacterial killing, in a separate group of 15 patients, incubation of cells from cirrhotic patients in control serum resulted in marked improvement of function compared to the results in autologous serum  $41\% \pm 4.2$  and  $12\% \pm 6$  respectively ( $p < 0.02$ ) suggesting that the difference is due to serum inhibitory factors. Similarly for chemotaxis there was a significant reduction in the cells from cirrhotics when preincubated in autologous serum, but if these were preincubated with control serum chemotaxis was not significantly different from the controls ( $34 \mu\text{m} \pm 5.6$  v  $37.4 \mu\text{m} \pm 4.2$ ).

The result for the production of NAG and  $\beta\text{G}$  are shown in Tables 3 and 4 which show values in supernatants and the cell button respectively and demonstrate the results of stimulation with endotoxin. In general, activities in supernatant and cells were decreased in cirrhotics compared to the controls. Differences were more marked for the NAG than for  $\beta\text{G}$ , where very low activities were recorded throughout. Results from 11 alcoholic patients without significant liver disease are also shown, and these values were not significantly different from control values. Table 5 shows the results for enzyme production divided into patients grouped on clinical grounds. Those with the severest disease, especially those with alcoholic hepatitis, were found to have the lowest activities, whilst those with quiescent disease were found to have near normal values. The small group of patients with cirrhosis on steroids were found to have slightly increased levels, although not statistically significant, and this group included most, but not all, the patients with chronic active hepatitis. Studying the chronic active group as

Table 3 Enzyme release by human monocytes measured in supernatant (mean (nmol/ $\mu$ g prot/h)  $\pm$ SEM)

	No of subjects	NAG		$\beta$ G	
		Unstimulated	Stimulated	Unstimulated	Stimulated
Controls	35	2.3 (0.26)	2.5 (0.291)	0.13 (0.06)	0.14 (0.012)
All cirrhotics	35	1.55 (0.3)*	1.8 (0.4)*	0.099 (0.03)**	0.124 (0.09)
Chronic active	10	2.4 (0.4)	2.4 (0.4)	0.11 (0.04)	0.12 (0.02)
Alcoholic + cirrhosis	15	1.2 (0.119)***	1.6 (0.28)**	0.079 (0.005)*	0.091 (0.009)
- cirrhosis	11	2.0 (0.35)	2.1 (0.39)	0.076 (0.01)	0.09 (0.016)
Other cirrhotics	10	1.2 (0.15)*	1.6 (0.25)	0.12 (0.03)	0.18 (0.05)
Obstructive jaundice	5	1.9 (0.24)	2.2 (0.28)	0.16 (0.04)	0.17 (0.02)

\*p &lt; 0.05 \*\*p &lt; 0.02 \*\*\*p &lt; 0.01.

Table 4 Enzyme cell content of monocytes (mean (nmol/ $\mu$ g prot/h)  $\pm$  SEM)

	No of subjects	NAG		$\beta$ G	
		Unstimulated	Stimulated	Unstimulated	Stimulated
Controls	35	0.48 (0.06)	0.52 (0.07)	0.09 (0.1)	0.11 (0.02)
All cirrhotics	35	0.36 (0.06)**	0.46 (0.06)	0.06 (0.01)*	0.085 (0.02)
Chronic active	10	0.49 (0.28)	0.64 (0.09)	0.06 (0.01)	0.06 (0.05)
Alcoholic + cirrhosis	15	0.23 (0.05)**	0.26 (0.05)***	0.06 (0.01)**	0.07 (0.01)
- cirrhosis	11	0.43 (0.08)	0.45 (0.11)	0.07 (0.01)	0.08 (0.01)
Other cirrhotics	10	0.39 (0.08)	0.69 (0.09)	0.06 (0.01)*	0.06 (0.01)
Obstructive jaundice	5	0.25 (0.08)	0.66 (0.28)	0.09 (0.015)	0.12 (0.05)

\*p &lt; 0.05 \*\*p &lt; 0.02 \*\*\*p &lt; 0.01.

Table 5 Production of enzymes by human monocytes (unstimulated) in patients grouped by severity of the disease (mean (nmol/ $\mu$ g prot/h)  $\pm$  SEM)

	No of subjects	NAG		$\beta$ G	
		Supernatant	Cells	Supernatant	Cells
Controls	35	2.3 (0.35)	0.48 (0.07)	0.13 (0.02)	0.09 (0.01)
Acute alcoholic hepatitis	4	1.3 (0.36)*	0.55 (0.1)	0.08 (0.017)	0.094 (0.03)
Active cirrhosis	10	1.34 (0.132)**	0.29 (0.06)**	0.079 (0.06)*	0.057 (0.024)
Quiescent cirrhosis	17	1.95 (0.27)	0.446 (0.115)	0.132 (0.029)	0.069 (0.012)
Cirrhosis on steroids	8	2.67 (0.5)	0.65 (0.25)	0.113 (0.016)	0.086 (0.02)

\*p &lt; 0.05 \*\*p &lt; 0.02.

Table 6 Effect of control and cirrhotic serum; portal and systemic specimens; and mixing experiments on monocytes obtained from a normal volunteer (mean  $\pm$  SEM)

	Chemotaxis ( $\mu$ m)	Bacterial killing (%)
Control serum (n = 20)	28.4 $\pm$ 3.2	17.4 $\pm$ 3.8
Cirrhotic serum (n = 20)	14 $\pm$ 4.5 (p < 0.05)	7.5 $\pm$ 3.5 (p < 0.02)
Mixture (n = 15)	16 $\pm$ 5.4 (p < 0.05)	8.4 $\pm$ 4.2 (p < 0.05)
Systemic serum (n = 18)	49.2 $\pm$ 2.7	65 $\pm$ 8.4
Portal serum (n = 18)	45.8 $\pm$ 2.2 (p < 0.05)	29.2 $\pm$ 4.2 (p < 0.02)
Mixture (n = 15)	46.2 $\pm$ 2.4 (NS)	34.6 $\pm$ 6.8 (p < 0.05)

The systemic/portal serum results were performed at different times and on different cells than the control/cirrhotic serum. Mixture refers to pooling of the relevant samples using equal volumes of each.

a whole, levels were not significantly below normal values.

A comparison of the effects of paired specimens of cirrhotic and control serum is shown in Table 6. Both chemotaxis and killing are significantly reduced by preincubation in cirrhotic serum compared to the controls. Also in this Table are shown the values for preincubation of normal monocytes with paired specimens of portal and systemic serum. Portal serum also results in significantly reduced chemotaxis and killing compared to the systemic serum. Mixing of cirrhotic with control and portal with systemic serum in equal volumes resulted in reduced chemotaxis and killing in the same range as those obtained for the cirrhotic and portal specimens respectively.

### Discussion

We have attempted to test various aspects of monocyte function in patients with cirrhosis and have demonstrated that this function is generally reduced. Although the changes are modest and do not always reach significance, results in cirrhotics are consistently reduced. Changes reached significance for chemotaxis, bacterial killing, monocyte spreading and bacterial phagocytosis. The number of monocytes present in cirrhotics was not different from the control population, but the results could be interpreted to suggest that the ability of these cells to mature into macrophages is less in cirrhotics than in the controls. Failure to show more clearcut differences between the cirrhotics and controls may be due to the inadequacies of currently available techniques. This reduced function appears to be mainly due to a serum inhibitor as incubating cirrhotic cells with normal serum resulted in some improvement in their function. Experiments in mixing cirrhotic and control serum showed that monocyte function was still reduced, and this would suggest the presence of an active inhibitor rather than a deficiency situation. Unfortunately the nutritional status of the patients was not assessed. It has previously been shown that there is defective bacterial opsonisation in patients with cirrhosis,<sup>26, 27</sup> but previous work has differed in respect to the nature of this defect. Some have found, as we have, that there appears to be an active inhibitor of chemotaxis and phagocytosis present.<sup>6-8</sup> Others, based on the results of mixing and dilution experiments, have suggested that a relative deficiency state exists, possible of complement, and that this accounts for the reduced opsonisation in cirrhotics.<sup>26</sup> In our view the reduced phagocytic function is unlikely to be simply due to a defect of opsonisation, as after preincubation with the appropriate serum the

actual phagocytosis was performed in fetal calf serum in these experiments. Similarly if this was a defect of opsonisation the defect would not be present on mixing the two sera.

Our results would suggest that the monocyte defect is secondary to the cirrhosis and not dependent on the underlying aetiology as it was present in all groups of cirrhotics to some extent. The enzyme production by the group of alcoholic patients, in whom there was no cirrhosis or hepatitis, was also not different from the controls. In chronic active hepatitis patients with cirrhosis, monocyte function was also reduced but all but one patient was taking corticosteroids, which, in the short-term at least, are known to depress monocyte function.<sup>28</sup> Long-term corticosteroid treatment has less effect on monocyte function, but we cannot exclude the possibility that the results obtained in our patients with chronic active hepatitis may be artificially lowered by the corticosteroid treatment, and that this may explain some of the differences obtained between our results and those previously obtained in a small group of patients in our laboratory.<sup>9</sup>

While most of the evidence suggests that the reduced function is due to the presence of an inhibitory factor, the NAG and  $\beta$ G production results might suggest that the cells themselves are abnormal, as these were performed in fetal calf serum. The production of these enzymes probably mirrors the ability of the monocytes to differentiate into macrophages<sup>29</sup> and further evidence to show there is a relative failure of maturation of these cells is obtained from the spreading experiments. Hassner recently reported<sup>6</sup> that treatment of cirrhotic monocytes with trypsin (to remove all traces of serum factors) resulted in a return of monocyte function to normal. Thus, although the cells were washed carefully, it is probable that these defects in enzyme production could also be accounted for by an effect of a serum factor carried over by the cells. However, we cannot exclude the possibility that there is a shift in the population of circulating monocytes in cirrhosis, although the findings of normal numbers of circulating monocytes which function normally in control serum makes this unlikely.

For enzyme production and NBT reduction, the monocytes were studied before and after stimulation either by endotoxin or latex and, in both cases, the response of the cells to this stimulation was not significantly different from that of the controls. Thus, the theory of hyperstimulated cells becoming immunosaturated and unable to react to fresh stimuli has not been substantiated and it would appear that these cells are capable of a normal response, at least in vitro. Endotoxaemia may be common in cirrhotics<sup>30, 31</sup> and assuming that the in

vivo effects are similar to the in vitro effects, a state of "monocyte activation" might have been expected. The relevance of the concept of the activated macrophage<sup>32</sup> is unknown but it is a term used to indicate a state of increased activity of the cells which occurs on exposure to various stimuli including endotoxin. Contrary to our expectation it appears that in cirrhosis the cells are less "activated" and that the serum contains "deactivating" substances. Activated macrophages also excrete collagenase<sup>33</sup> and thus this mechanism may be important in the instigation or perseverance of increased collagen deposition in the liver and hence the development of cirrhosis.

We have shown that normal cells exposed to portal vein serum show reduced chemotaxis and bacterial killing compared to those exposed to simultaneously taken systemic serum specimens. The mixing experiments would also suggest that there is an active inhibitor present. Thus, rather than the monocyte defect being explained on the basis of a constant stimulation by antigens resulting in a failure to respond to fresh stimulation, our results would suggest that inhibitory substances (probably gut-related antigens) bypass, or are not sufficiently removed by, the failing Kupffer cells in the cirrhotic liver. We have further investigated this by measuring immune complexes and endotoxin in the portal systemic serum and comparing them to the effect of the individual specimen on the monocyte responses. These studies suggest that it is immune complexes rather than endotoxin which have the most marked effect (Holdstock *et al* submitted for publication).

Ferluga<sup>34</sup> has suggested that monocytes, which are presumed to be Kupffer cell precursors, may be important in certain types of liver damage. He has described an animal model where mice, primed with *Corynebacterium parvum* and exposed to endotoxin, developed acute liver failure. He suggests that monocytes recruited into the liver by the *Corynebacterium parvum* may be activated by the endotoxin to release potentially toxic substances. He further suggests that the same mechanism may be operative in hepatitis. We have recently provided further evidence for this from in vitro studies in rats<sup>35</sup> but contrary to our original expectation<sup>9</sup> that enzyme release from monocytes might be increased in cirrhosis, and thus a possible indicator of active liver damage, the finding that enzyme release is reduced does not support this theory of hepatocyte damage in the patient groups studied. Crucial to this is the unanswered question regarding the relation of peripheral monocyte function to that of Kupffer cells. If such a relation exists our findings suggest that Kupffer cell function is reduced in cirrhotics. Thus the Kupffer cells may be unable to protect the

hepatocyte from further damage. This finding is in keeping with recent in vivo studies of Kupffer cells activity<sup>36</sup> suggesting reduced function in cirrhosis.

In conclusion, we have demonstrated wide-ranging defects in monocyte function in patients with cirrhosis. This appears to be secondary to the presence of cirrhosis and is probably not of primary immunopathogenic importance. The abnormality may be due to the presence of an active inhibitor which possibly originates from the portal venous system due to failure of the filtering mechanism of the normal liver. The defects in monocyte function together with previously shown defects in neutrophil function and other abnormalities including defective serum bactericidal activity,<sup>37</sup> may all play a role in the susceptibility of these patients to infection. It is possible that nutritional deficiencies<sup>38</sup> in part explain the abnormalities, but this would not seem to explain the presence of an active inhibitor substance.

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