

*Liver and biliary***Neutrophil adherence in chronic liver disease and fulminant hepatic failure**

M ALTIN, I A RAJKOVIC, R D HUGHES, AND ROGER WILLIAMS

From the Liver Unit, King's College Hospital and Medical School, Denmark Hill, London

SUMMARY Abnormal adherence of neutrophils to nylon fibre *in vitro* was found in blood from 17 of 51 (33.3%) patients with chronic or acute liver disease of different aetiologies. Patients with chronic liver disease had a much wider range of values than the controls and the sub-group with alcoholic cirrhosis had significantly higher adherence ($72.4 \pm \text{SD } 6.2\%$) than that of controls ($65.8 \pm \text{SD } 5.2\%$). The patients with chronic active hepatitis ($68.2 \pm 12.7\%$) or primary biliary cirrhosis ($69.2 \pm 6.6\%$) were not different from controls. Significantly reduced neutrophil adherence ($56.2 \pm 8.7\%$) was found in blood from patients with fulminant hepatic failure. These abnormalities in neutrophil adherence may be due to the effects of the split components of serum complement and dependent on the degree and duration of exposure of the neutrophils. Defects in neutrophil adherence may in part contribute to the increased susceptibility to infection in patients with acute and chronic liver disease.

The neutrophil response to an acute inflammatory stimulus involves a complex sequence of events which lead to migration of the cells to the extra-vascular compartment with phagocytosis at the site of inflammation.¹ Adherence of neutrophils to the vascular endothelium is an early event in response to activated serum complement and is required before migration into tissue.² MacGregor *et al*³ have developed a technique in which neutrophil adherence to nylon fibres packed into glass pasteur pipettes is determined. A direct relationship between the values obtained and the adherence of neutrophils to endothelial cells was found.⁴ Abnormal neutrophil adherence has been described in chronic alcoholics,⁵ and defects in neutrophil chemotaxis have been reported in patients with chronic⁶⁻¹⁰ or acute liver disease (unpublished findings). These defects may contribute to the increased susceptibility of these patients to bacterial infection.¹¹⁻¹⁶

We have determined neutrophil adherence by means of an improved nylon fibre technique in a series of patients with fulminant hepatic failure and with compensated chronic liver disease.

Methods**PATIENTS**

Blood samples were obtained from 38 patients with compensated chronic liver disease, which, on the basis of clinical, laboratory and histological findings, was attributed to alcoholic cirrhosis in 16, chronic active hepatitis in 12 (in four of whom cirrhosis was already present), and primary biliary cirrhosis in 10. Thirteen patients with fulminant hepatic failure in grade III or IV encephalopathy were also studied, the cause being paracetamol overdose in eight and viral hepatitis in the others. All 13 patients were receiving 10% dextrose and none received fresh frozen plasma or blood during the period of study. Patients with proven bacterial infection at the time of the study or those in whom infection subsequently developed were excluded. Fifteen normal healthy subjects working in the laboratory acted as controls (Table 1).

All blood samples were collected into syringes containing preservative free heparin (Leo Laboratories Ltd, Hayes, Middlesex) to give a final concentration of 10 U/ml. Neutrophil adherence was determined immediately.

NEUTROPHIL ADHERENCE ASSAY

The method used was based on that developed by Stecher and China¹⁷ to study the effect of anti-

Address for correspondence: Dr Roger Williams, Liver Unit, King's College Hospital, Denmark Hill, London SE5 8RX
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Table 1 Relation of neutrophil adherence to peripheral WBC count, aetiology, and outcome in 13 patients with fulminant hepatic failure

Case No.	Aetiology	Outcome	WBC count/ μ l		
			Total	Neutrophils	% adherence
1	Paracetamol	died	12000	10440	48
2	Paracetamol	died	3600	2664	55
3	Paracetamol	alive	7400	6290	43.8
4	Hepatitis B	alive	22000	16060	58.4
5	Hepatitis B	died	10500	9135	62.8
6	Non A/B	alive	9000	6480	57.9
7	Hepatitis A	alive	17200	15308	72.9
8	Paracetamol	alive	16500	11880	52.9
9	Paracetamol	died	5000	4700	40.6
10	Paracetamol	died	16400	14104	58.3
11	Paracetamol	died	4000	3480	53.7
12	Paracetamol	died	11300	8814	60.9
13	Paracetamol	died	4500	4095	64.9
Normal range				2650-6386	55.4-76.2

inflammatory agents on the adherence of rat neutrophils. To establish the optimum packing weight of nylon fibre to use with human neutrophils, individual 1 ml disposable tuberculin syringes (Beckton-Dickinson Plastipak, Wembley, Middlesex) were packed with either 20, 40, 60 or 80 mg of scrubbed nylon fibre (3 denier, 1.5 inch, type 200, Fenwal, Travenol Laboratories, Thetford, Norfolk) to the 0.1, 0.15, 0.2, and 0.25 ml mark of the syringe respectively. The packed columns were fitted with a three way tap and a 25 gauge $\frac{3}{8}$ inch disposable needle (Gillette Surgical, Isleworth, Middlesex), supported vertically, and incubated at 37°C for 10 minutes. Three columns were used for each sample and 1 ml of heparinised venous blood applied to each column with a 10 ml disposable syringe. After a further five minutes incubation at 37°C the taps were opened and the blood allowed to filter through the nylon for 10 minutes. Aliquots of blood were incubated in plastic tubes for the same period for the unfiltered sample. Total and differential leucocyte counts were measured in triplicate on each sample using an electronic counter (Coulter Model ZF, Luton, Bedfordshire) and visual counts on stained blood films respectively.

The neutrophil adherence was calculated from the following equation:

$$\% \text{ Adherence} = 100 - \left(\frac{\text{neutrophils in effluent blood}}{\text{neutrophils in unfiltered samples}} \right) \times 100$$

The effect of the different packing weights of nylon fibre on the adherence of normal neutrophils is shown in Fig. 1. The 20 mg was chosen for the patient samples as it gave a mid-range adherence value which would enable detection of enhanced as

well as reduced adherence of neutrophils.

Results

Seventeen patients (33.3%) had abnormal neutrophil adherence, defined as being outside two standard deviations of the normal range (Fig. 2).

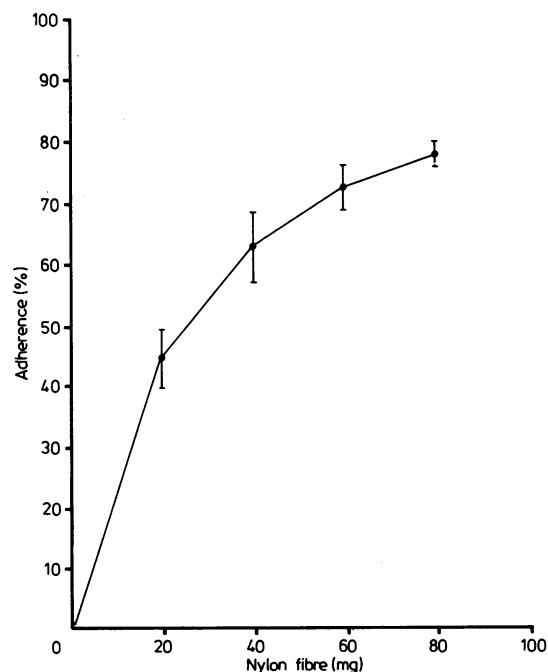


Fig. 1 Mean neutrophil adherence in normal controls with various packing weights of nylon fibre (\pm SD).

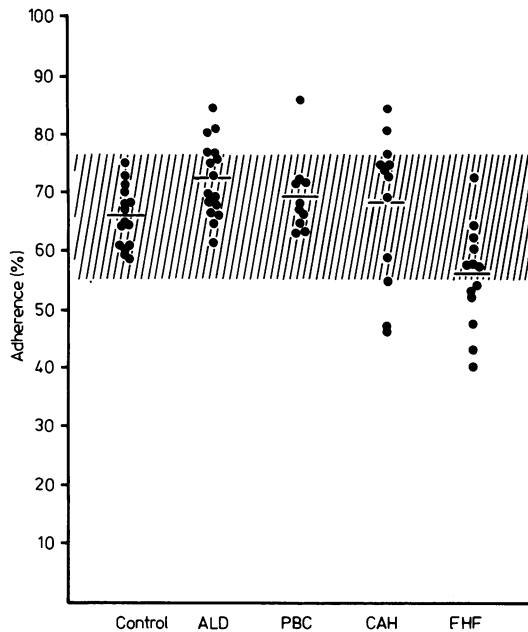


Fig. 2 Neutrophil adherence in different groups of patients studied. Shaded area represents values ± 2 standard deviations of the normal range.

Increased adherence was found in five of the 16 patients with alcoholic cirrhosis, three of the 12 patients with chronic active hepatitis, and one of the 10 patients with primary biliary cirrhosis. The mean value of neutrophil adherence in alcoholic cirrhosis ($72.4 \pm \text{SD } 6.2\%$) was significantly higher ($p < 0.02$ Wilcoxon's rank test) than that of the control subjects ($65.8 \pm 5.2\%$), whereas for the patients with chronic active hepatitis or primary biliary cirrhosis, with mean values of $68.2 \pm 12.7\%$ and $69.2 \pm 6.6\%$ respectively, the difference was not statistically significant. Three of the patients with chronic active hepatitis were taking prednisolone (2.5–10 mg/day) at the time of the study and one of these had neutrophil adherence outside the normal range. The age or sex of the patients had no relationship with neutrophil adherence as similar frequency of abnormalities was recorded on older and younger patients of either sex.

Reduced neutrophil adherence was detected in six of the 13 patients with fulminant hepatic failure, in all of whom the cause of hepatic failure was paracetamol overdose. The mean neutrophil adherence in these patients ($56.2 \pm 8.7\%$) was significantly lower ($p < 0.01$ Wilcoxon's rank test) than that of the control subjects.

Peripheral neutrophil counts (Table 2) were

significantly raised ($p < 0.05$) in patients with fulminant hepatic failure but were normal in the other groups studied. The percentage of neutrophils in the total count was significantly higher in patients with alcoholic cirrhosis ($p < 0.01$), primary biliary cirrhosis ($p < 0.05$) or fulminant hepatic failure ($p < 0.01$) than that in controls, but there was no correlation between the adherence value and either the absolute number or the percentage of neutrophils in any of the groups of patients. Neither was there any significant relation between the changes in neutrophil adherence and the severity of the liver damage as assessed by biochemical and histological findings.

Discussion

MacGregor found a reciprocal relationship between peripheral neutrophil count and neutrophil adherence value,¹⁸ but this was not the case in the present study. Although the patients with fulminant hepatic failure had reduced neutrophil adherence with raised neutrophil counts, the patients with chronic liver disease with an increased neutrophil adherence did not have lower neutrophil counts.

The study of Wozniak and Silverman⁵ reports increased neutrophil adherence in chronic alcoholics 12–48 hours after their withdrawal from alcohol and suggest that the increase in neutrophil adherence above that of normal controls was a 'rebound' phenomenon. In our study, increased neutrophil adherence was predominantly associated with alcoholic cirrhosis but these patients were tested at least 72 hours after admission to hospital to preclude these effects. Thus the increased adherence found in our patients with alcoholic cirrhosis should reflect the effects of liver impairment. The abnormality was not restricted to alcoholic liver disease, being found in patients with chronic active hepatitis as well. The increased adherence of neutrophils in chronic liver

Table 2 Mean counts and percentages of neutrophils in various groups of patients investigated

	No.	Neutrophils/ μl		% Neutrophils (mean \pm SD)
		Mean	Range	
Alcoholic liver disease	16	4625	972–10250	73.3 \pm 10
Chronic active hepatitis	12	4336	1976–8820	65.9 \pm 9.2
Primary biliary cirrhosis	10	3814	2240–10384	69.1 \pm 9.3
Fulminant hepatic failure	13	8727	2664–16060	82.7 \pm 9.1
Normal controls	15	4052	2650–6386	58.0 \pm 6.3

disease may be a reflection of their exposure *in vivo* to low grade complement activation generated by gut derived antigens¹⁹ or immune complexes²⁰ entering the serum of these patients. Craddock *et al*²¹ have shown that the split products of complement, particularly C_{5a} fragment, increase neutrophil adherence and their tendency to aggregate. Reduced neutrophil adherence was found in patients with fulminant hepatic failure who also have defective neutrophil locomotion,²² a step that closely follows adherence of the cells to the vascular endothelium. Direct effects of paracetamol are excluded as residual blood concentrations in our patients were virtually undetectable. Our findings suggest that the defect in neutrophil locomotion may be because of a reduced capacity of the neutrophils to respond to serum mediators. Patients with fulminant hepatic failure have extremely low concentrations of serum complement,²³ probably as a result of both reduced synthesis by the damaged liver and increased consumption; the latter may reflect the magnitude of the immunological stimulation to which the neutrophils are exposed and which may lead to their deactivation.

It may seem contradictory to attribute both increased and decreased neutrophil adherence to the effects of complement, but the response of the neutrophil depends largely on the concentration of these serum factors. Hence exposure of neutrophils to activated complement, while giving rise to increased adherence and other functions initially, may ultimately result in transient or even permanent deactivation of the cells, analogous to the *in vitro* neutrophil deactivation,²⁴ preventing their response to subsequent stimulation.

The pattern of neutrophil adherence in patients with chronic or acute liver disease probably represents different stages in the neutrophil response to the split products of complement and may reflect the severity and time course of the disease. The abnormalities in neutrophil adherence reported here may in part contribute to the increased incidence of chemotactic defects which have also been shown⁵⁻¹⁰ and which may be partly responsible for the increased susceptibility of patients with liver disease to infection.

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