Liver and biliary

Neutrophil adherence in chronic liver disease and fulminant hepatic failure

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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±SD 6.2%) than that of controls (65.8±SD 5.2%). The patients with chronic active hepatitis (68.2±12.7%) or primary biliary cirrhosis (69.2±6.6%) were not different from controls. Significantly reduced neutrophil adherence (56.2±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 al3 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,2 and defects in neutrophil chemotaxis have been reported in patients with chronic6-10 or acute liver disease (unpublished findings).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 Chinea17 to study the effect of anti-
Neutrophil adherence in chronic liver disease and fulminant hepatic failure

Table 1  Relation of neutrophil adherence to peripheral WBC count, aetiology, and outcome in 13 patients with fulminant hepatic failure

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Aetiology</th>
<th>Outcome</th>
<th>WBC count/μl</th>
<th>Neutrophils</th>
<th>% adherence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Neutrophils</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Paracetamol</td>
<td>died</td>
<td>12000</td>
<td>10440</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>Paracetamol</td>
<td>died</td>
<td>3600</td>
<td>2664</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>Paracetamol</td>
<td>alive</td>
<td>7400</td>
<td>6290</td>
<td>43.8</td>
</tr>
<tr>
<td>4</td>
<td>Hepatitis B</td>
<td>alive</td>
<td>22000</td>
<td>16060</td>
<td>58.4</td>
</tr>
<tr>
<td>5</td>
<td>Hepatitis B</td>
<td>died</td>
<td>10500</td>
<td>9135</td>
<td>62.8</td>
</tr>
<tr>
<td>6</td>
<td>Non A/B</td>
<td>alive</td>
<td>9000</td>
<td>6480</td>
<td>57.9</td>
</tr>
<tr>
<td>7</td>
<td>Hepatitis A</td>
<td>alive</td>
<td>17200</td>
<td>15308</td>
<td>72.9</td>
</tr>
<tr>
<td>8</td>
<td>Paracetamol</td>
<td>alive</td>
<td>16500</td>
<td>11880</td>
<td>52.9</td>
</tr>
<tr>
<td>9</td>
<td>Paracetamol</td>
<td>died</td>
<td>5000</td>
<td>4700</td>
<td>40.6</td>
</tr>
<tr>
<td>10</td>
<td>Paracetamol</td>
<td>died</td>
<td>16400</td>
<td>14104</td>
<td>58.3</td>
</tr>
<tr>
<td>11</td>
<td>Paracetamol</td>
<td>died</td>
<td>4000</td>
<td>3480</td>
<td>53.7</td>
</tr>
<tr>
<td>12</td>
<td>Paracetamol</td>
<td>died</td>
<td>11300</td>
<td>8814</td>
<td>60.9</td>
</tr>
<tr>
<td>13</td>
<td>Paracetamol</td>
<td>died</td>
<td>4500</td>
<td>4095</td>
<td>64.9</td>
</tr>
</tbody>
</table>

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 ½ 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:

\[
\text{% Adherence} = 100 - \frac{\text{neutrophils in effluent blood}}{\text{neutrophils in unfiltered samples}} \times 100
\]

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 (±SD).](http://gut.bmj.com/ on January 7, 2018 - Published by group.bmj.com)
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±SD 6.2%) was significantly higher (p<0.02 Wilcoxon's rank test) than that of the control subjects (65.8±5.2%), whereas for the patients with chronic active hepatitis or primary biliary cirrhosis, with mean values of 68.2±12.7% and 69.2±6.7% 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±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, 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 Silverman 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

<table>
<thead>
<tr>
<th>Disease</th>
<th>No.</th>
<th>Neutrophils/μl</th>
<th>% Neutrophils (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic liver disease</td>
<td>16</td>
<td>4625</td>
<td>972–10250</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>12</td>
<td>4336</td>
<td>1976–8820</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>10</td>
<td>3814</td>
<td>2240–10384</td>
</tr>
<tr>
<td>Fulminant hepatic failure</td>
<td>13</td>
<td>8727</td>
<td>2664–16060</td>
</tr>
<tr>
<td>Normal controls</td>
<td>15</td>
<td>4052</td>
<td>2650–6386</td>
</tr>
</tbody>
</table>

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