

Circulating immune complexes in patients with fulminant hepatic failure

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SUMMARY Circulating immune complexes were found in 15 of 16 patients with fulminant hepatic failure due to viral hepatitis and all of six patients who had had halothane anaesthesia; however, they were found in only five of 32 patients with paracetamol-induced hepatic necrosis. The levels of circulating immune complexes were not related to the severity of the clinical course, development of renal failure, final outcome, or severity of hypocomplementaemia. All the patients had depressed reticuloendothelial function as assessed by the clearance of ^{125}I microaggregated albumin. These findings show that circulating immune complexes in fulminant hepatic failure are not simply a reflection of an immune response to liver antigens released as a result of the hepatic necrosis; nor are they a reflection of the failure of the reticuloendothelial system. This supports the view that circulating immune complexes are associated with immune mediated liver injury and may contribute to the process.

The mechanism responsible for the progression of some cases of acute viral hepatitis to fulminant hepatic failure is poorly understood. In fulminant hepatic failure due to hepatitis B virus the early appearance of antibody to hepatitis B surface antigen (HBsAg) and rapid disappearance of HBsAg from the serum,^{1,2} and the finding of immune complexes on direct electron-microscopy,³ have led some authors to postulate that severe liver damage may follow the deposition of immune complexes in the liver sinusoids.² Whether or not immune complexes play a part in other types of fulminant viral hepatitis or in drug-induced hepatic failure has not been investigated.

In this study we measured levels of circulating immune complexes in patients with fulminant viral hepatitis and in patients with fulminant hepatic failure after halothane anaesthesia or paracetamol overdose. The level of circulating immune complexes depends on a balance between formation and removal from the circulation by the reticuloendothelial system;⁴ changes in the latter were assessed by measuring the clearance from the circulation of iodinated microaggregated human albumin (^{125}I MAA).⁵ As immune complexes consume complement and their deposition in the glomeruli of the kidney can cause renal failure,⁶ which is common in fulminant hepatic failure,⁷ serum

complement activity and renal function were also assessed to determine the immunological and clinical significance of the circulating immune complexes.

Methods

PATIENTS

Fifty-five patients with fulminant hepatic failure were studied (28 female, 27 male), aged from 14 to 67 years (median 28 years), all of whom had deteriorated to grade IV hepatic encephalopathy.⁸ Hepatic necrosis was attributed to paracetamol self-poisoning in 32 patients; viral type A in six (shown by the presence of the specific antibody with ELISA technique—Organon Teknika); hepatitis type B in three (shown by the presence of HBsAg with ELISA (Ausyme)); presumed non-A, non-B hepatitis in seven; and halothane-associated hepatitis in seven. All patients received full supportive care⁹ and daily periods of haemodialysis with a polyacrylonitrile membrane (Rhone Poulenc RP 6) and/or haemoperfusion with a Haemacol 100 column (Smith and Nephew Research Ltd) and added prostacyclin as platelet protective agent.¹⁰ The initial measurements were always made before such periods of temporary liver support.

MEASUREMENT OF IMMUNE COMPLEXES AND COMPLEMENT LEVELS

Venous blood was allowed to clot for two hours at

room temperature; serum was separated and was used either immediately or stored at -70°C or -196°C until time of assay. Samples were thawed only once. Levels of circulating immune complexes were measured by the Cl_q binding assay according to the method of Zubler *et al.*¹¹ Normal range was calculated from the mean ± 2 SD of values obtained from the sera of 25 blood donors.

Functional activities of total haemolytic complement (CH_{50}) and total alternative pathway were measured in 20 patients by haemolytic diffusion in agarose.^{12,13} Concentrations of immunoreactive C3 were determined by radial immunodiffusion against rabbit antiserum (kindly provided by Professor D K Peters). Results were expressed as a percentage of the activity of a control serum pool, derived from six healthy laboratory personnel, which was included as a standard in each plate.

ASSESSMENT OF RETICULOENDOTHELIAL SYSTEM FUNCTION

Microaggregated iodinated serum albumin (^{125}I MAA) was prepared by the method of Iio and Wagner⁵ as previously reported.¹⁴ After an intravenous injection of sodium iodide to block radio-iodine uptake by the thyroid, ^{125}I MAA, 5 mg/kg body weight was injected; 2 ml blood samples were taken into EDTA anticoagulant 0, 3, 5, 7, 10, 12, 15, and 20 minutes later. The plasma was separated immediately and the ^{125}I activity in a 0.5 ml aliquot was counted with a gamma counter (Hewlett Packard). The half life ($t_{1/2}$) of clearance was calculated by plotting \log_{10} counts per minute against time in minutes. Four normal volunteers acted as controls.

Results

Fifteen of the 16 patients with fulminant hepatic failure attributed to viral hepatitis had raised levels of circulating immune complexes, with a mean for the 16 patients of $33.6 \pm \text{SD } 14.3\%$, which was significantly ($P < 0.01$, Wilcoxon's rank sum test) higher than the mean of 9.6 ± 3 for controls (Figure). The raised level of circulating immune complexes in patients with viral hepatitis was not related to the type of viral hepatitis, to the severity of the clinical course, or final outcome. The six patients with halothane-associated hepatitis also had higher levels of circulating immune complexes than controls, with a mean of $31.4 \pm \text{SD } 14.5$, which was similar to that in patients with viral hepatitis (Figure). In contrast, 27 of the 32 patients with paracetamol overdose had normal levels, with a mean for the 32 patients of $13.5 \pm \text{SD } 3.4$. Five patients had raised levels; the two with the highest values had received transfusions of plasma protein fraction within the preceding 12 hours, and one of these two

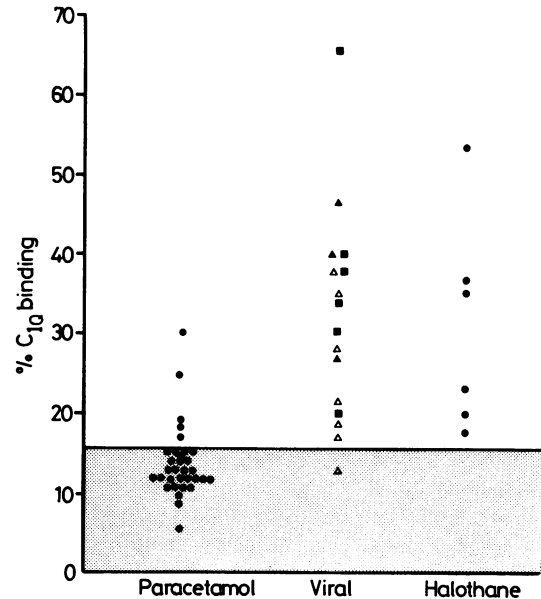


Figure Initial measurement of Cl_q binding activity in patients with fulminant hepatic failure. \blacktriangle hepatitis B, \blacksquare hepatitis A, \blacktriangle presumed non-A, non-B. Dotted area: normal range mean ± 2 SD.

patients also had an *E. coli* septicaemia. Similarly, the patient with the highest levels of immune complexes in the halothane-associated hepatitis group had pneumonia. Except for these three patients, no other patients in the series had evidence of bacterial infection or had received blood products before the first sample was taken. Serial studies of circulating immune complexes showed that, in patients with viral and halothane-associated hepatitis, levels remained raised. Similarly, in patients with paracetamol overdose, levels remained within the normal range unless complicated by the development of culture-proven bacterial infection or administration of blood products, which in three patients was associated with a mean increase of 220% of initial levels.

Nine patients had renal failure (creatinine >350 $\mu\text{mol/l}$ or urine output ≤ 300 ml/24 h) during the study, and in 14 others it developed later. There was no difference in levels of circulating immune complexes between patients who did or did not develop renal failure, or in whom it had already developed.

SERUM COMPLEMENT AND RETICULOENDOTHELIAL FUNCTION

The functional activity of the alternative and classical pathways (CH_{50}) of complement and levels of immunoreactive C3 were reduced in all 20 patients

studied to a mean of less than 30% of the activity of the control serum pool (Table). The severity of the complement deficiency was similar in patients with viral hepatitis, halothane-associated hepatitis, and paracetamol overdose and there was no correlation between the level of serum complement and that of circulating immune complexes.

The plasma half-life ($t_{1/2}$) of $^{125}\text{IMAA}$ was prolonged in all 25 patients studied, ranging from 18 to 36 minutes compared with an upper limit of normal for controls of 15.6 (mean \pm 2 SD). There was no difference in the degree of prolongation of $t_{1/2}$ between any of the patient groups; and no correlation was found between the $t_{1/2}$ and either levels of circulating immune complexes or complement levels.

Table Serum complement expressed as percentage activity of control serum pool (mean \pm SD)

Clinical groups	Number of cases	C3	Classical pathway CH_{50}	Total alternative pathway
All cases	20	25.4 \pm 14.5	9.3 \pm 13.2	23.8 \pm 13.1
Paracetamol	9	29.3 \pm 16.9	18 \pm 15.1	26.5 \pm 9.8
Viral hepatitis	7	33 \pm 17.5	19.2 \pm 13.3	23.1 \pm 9.6
Halothane	3	15.6	10.6	14.4
Controls	30	103 \pm 18.5	108 \pm 19	107 \pm 20

Discussion

As, with few exceptions, levels of circulating immune complexes were not raised in patients with paracetamol overdose it is unlikely that the increased levels in cases attributed to viral or halothane-associated fulminant hepatic failure are simply a reflection of the immune response to released liver antigens as a result of the hepatic necrosis. It is more likely that antibody, produced in response to specific antigens generated by the aetiological agent, complexes with the corresponding antigen released from the damaged liver cells. Specific immunological responses to neo-antigens occur in acute hepatitis A and B virus infections and are likely to accompany infection with non-A, non-B agent(s). Although the mechanism of halothane-associated hepatic necrosis has been the subject of much debate, a recent study has shown that serum from most of these patients contains antibody to a neo-antigen formed on the surface of rabbit hepatocytes.¹⁵ The formation of this antigen is associated with the metabolic degradation of halothane *via* an oxidative pathway.¹⁶ This is the first time increased levels of circulating complexes have been reported in cases of fulminant halothane-

associated hepatitis and is additional evidence in favour of an immunological mechanism. However, the finding of increased levels of circulating immune complexes in patients with fulminant viral and halothane-associated hepatitis may be only one aspect of a number of immunological aberrations in these two disorders and not necessarily an indication of an aetiological role for the immune complexes in the production of the liver damage.

In cases of paracetamol overdose, hepatic necrosis is dose dependent and is thought to be due to the formation of reactive intermediary metabolites. In these patients levels of circulating immune complexes were raised only in association with episodes of infection or transfusion and it is likely that these complexes are manifestations of immune responses to bacterial antigens and altered plasma proteins, respectively. Methods of measurement of circulating immune complexes all have drawbacks, in particular the binding of C1q by DNA, bacterial lipopolysaccharide, and heparin which may produce erroneous results. However, with the technique of Zubler *et al.* (1976)¹¹ only heparin is a potential problem and, as each of our patient groups received similar amounts, this is unlikely to be a source of significant error. As sera from all the patient groups were handled in the same way with respect to collection, separation, and storage, variations of aggregation of immunoglobulin or formation of cryoprecipitates are unlikely to account for the difference in C1q binding activity between the different patient groups.

The severe impairment of the function of the reticuloendothelial system shown by the prolonged clearance from the circulation of $^{125}\text{IMAA}$ in all the patients with fulminant hepatic failure, irrespective of aetiology, has several important implications. Such a defect would impair the clearance from the circulation of circulating immune complexes, bacteria, and endotoxins, which has been shown in animal studies to contribute to liver damage.¹⁷ Both parenchymal and non-parenchymal liver cells are thought to be the site of synthesis of many complement factors, and with massive liver cell necrosis the production of these will be reduced. This almost certainly explains the low levels of complement found in all patients studied, including those in whom circulating immune complexes were not increased. As complement turnover studies cannot be performed *in vivo*, because of the short duration of illness and unstable clinical course, the importance of complement consumption cannot be assessed. Serum complement deficiency may contribute to the increased levels of circulating immune complexes, as there is evidence that the alternative pathway of complement is involved in the solubilisation of immune complexes.¹⁸

Renal failure has been attributed to immune

complex deposition in some cases of acute viral hepatitis, especially type B, but in our patients there was no correlation between levels of circulating immune complexes and the occurrence of renal failure. Furthermore, renal failure occurred with equal frequency in cases of fulminant hepatic failure attributed to both paracetamol overdose or viral hepatitis, although levels of circulating immune complexes were normal in the former. It is more likely that the impairment of the reticuloendothelial system predisposes to the development of systemic endotoxaemia with consequent renal vasoconstriction and renal failure.¹⁹

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