

## Serological markers in fulminant hepatitis B

A E S GIMSON, R S TEDDER, Y S WHITE,  
A L W F EDDLESTON, AND ROGER WILLIAMS

*From The Liver Unit, King's College Hospital and Medical School, Denmark Hill, London, and The Department of Virology, Middlesex Hospital Medical School, London.*

**SUMMARY** Serological markers for hepatitis B virus infection have been examined in 34 patients with acute hepatitis B, 17 of whom developed fulminant hepatic failure. Hepatitis B surface antigen concentrations were significantly lower and hepatitis Be antigen was less frequently detectable in patients with fulminant hepatic failure compared with those with acute hepatitis (median 0.64  $\mu\text{g}$ , range 16-0 and median 32  $\mu\text{g}$  and range 100-4  $\mu\text{g}$  respectively,  $p < 0.001$ ; HBeAg detected in 12% and 88% respectively,  $p < 0.001$ ). The IgM component of hepatitis B core antibody was significantly higher in the patients with fulminant hepatic failure with median values of 500 IU/ml compared with those with uncomplicated hepatitis (median 202 IU/ml,  $p < 0.05$  Wilcoxon's rank test). Three patients who developed a fulminant course had detectable levels of either anti-HBs or anti-HBe. These results are consistent with enhanced antibody responses to all three hepatitis B virus antigens and more rapid clearance of the latter during fulminant hepatic failure.

Why acute viral hepatitis should occasionally pursue a fulminant course is poorly understood. In hepatitis B virus (HBV) infections there is evidence of an enhanced antibody response to the surface antigen (HBsAg) with a more rapid appearance of anti-HBs in blood and a shorter period of HBs antigenaemia in patients with a fulminant course compared with those with uncomplicated hepatitis.<sup>1,2</sup> It has been suggested that this enhanced antibody production may cause an Arthus reaction in hepatic sinusoids resulting in ischaemic necrosis of hepatocytes.<sup>2</sup> Whether these findings represent an exaggerated response to a single antigenic determinant of the HBV or are part of a wider heightened responsiveness to the other HBV antigens is not clear. To investigate this we have measured HBsAg, anti-HBs, hepatitis Be antigen (HBeAg) and its antibody (anti-HBe) as well as the early response to the hepatitis B core antigen (HBcAg) by assaying for the specific IgM antibody (anti-HBc IgM) in serum from 34 patients with acute hepatitis including 17 patients who developed a fulminant course.

### Methods

#### PATIENTS

Seventeen of the 34 patients investigated developed severe hepatic dysfunction and Grade IV encephalopathy within eight weeks of the onset of symptoms, and fulfilled established criteria for the diagnosis of fulminant hepatic failure.<sup>3</sup> The remaining 17 patients with acute hepatitis B were never encephalopathic and presented with jaundice. The two groups were comparable in age, but there were more female patients in the fulminant hepatic failure group (11 of 17 compared with four of 17 respectively).

All serological tests were performed on samples drawn within nine days of the onset of jaundice. HBsAg was detected by a solid-phase radioimmunoassay (RIA, nominal sensitivity 0.5 ng/ml for subtypes ad and ay) and expressed in  $\mu\text{g}$  HBsAg/ml;<sup>4</sup> anti-HBs was detected by a solid-phase RIA.<sup>5</sup> Sera reactive for this antibody were quantified by reference to the WHO anti-HBs standard and expressed in International Units per ml (IU/ml). HBeAg and anti-HBe were also detected by a solid-phase RIA.<sup>5</sup> The specificity of reactivity for HBeAg was confirmed by neutralisation with anti-HBe. Anti-HBc IgM was detected by a solid phase

Address for correspondence: Dr Roger Williams, Liver Unit, King's College Hospital Medical School, Denmark Hill, London SE5 8RX.

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IgM antibody capture RIA (MACRIA). Sera containing anti-HBc IgM were quantified by reference to a standard serum containing a high level of this antibody, and expressed as arbitrary units per ml.<sup>6</sup>

#### STATISTICS

Results are given as mean  $\pm$  SEM or median and range if the data are non-parametric. Analysis was carried out by the Mann Whitney sum rank test or chi square test where appropriate.

#### Results

Sera from patients with uncomplicated hepatitis were all HBsAg positive whereas in the group with fulminant hepatic failure two patients were HBsAg negative ( $<0.5$  ng/ml). The concentrations of HBsAg in the group with fulminant hepatic failure were significantly lower than those with uncomplicated hepatitis (median  $0.64$   $\mu$ g, range  $16-0$   $\mu$ g, and median  $32$   $\mu$ g and range  $100-4$   $\mu$ g, respectively,  $p < 0.001$ ). In the fulminant hepatic failure group the two patients who were HBsAg negative had detectable anti-HBs in the serum. In contrast, anti-HBs was not found in any of the admission samples from patients with uncomplicated hepatitis.

HBeAg was present in the admission sample from only two of the 17 patients with fulminant hepatic failure (12%) but was found in all but two (88%) admission samples from the 17 patients with acute hepatitis ( $p < 0.001$ ). Anti-HBe was present in sera from two patients with fulminant hepatic failure. In one, the HBsAg concentration was low (40 ng/ml) and in the other, which was one of the two HBsAg-negative sera, anti-HBs was already also present. In contrast, anti-HBe was not detected in the admission sample of any patient with uncomplicated acute hepatitis.

All 34 patients had diagnostic levels of anti-HBc IgM though the median levels were significantly higher in the fulminant hepatic failure group, 500 arbitrary IU/ml, compared with the group with uncomplicated hepatitis, 202 arbitrary IU/ml ( $p < 0.05$ , Wilcoxon's rank test, Figure). In the group with fulminant hepatic failure there was no correlation between the level of anti-HBc IgM and age, severity of liver damage as judged by maximum prolongation of prothrombin time, or eventual outcome.

#### Discussion

Low concentrations of HBsAg and the rapid appearance of anti-HBs in patients with fulminant hepatic failure are in agreement with results

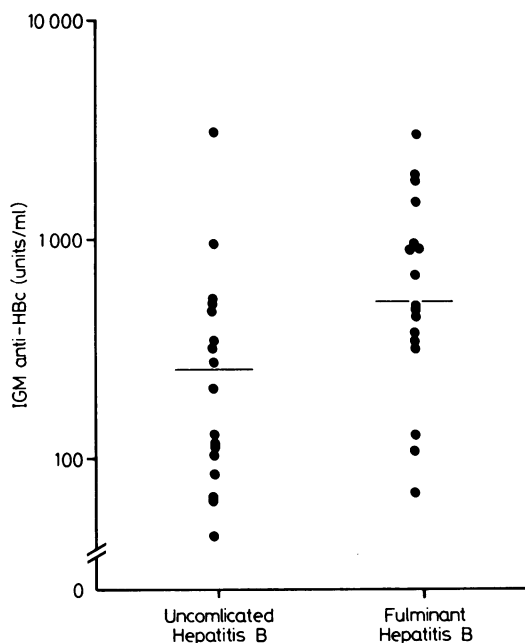


Figure Anti-HBc IgM concentrations (log IU/ml) and median in patients with acute viral hepatitis and fulminant hepatic failure.

reported in earlier series.<sup>1,2</sup> In uncomplicated hepatitis the presence of HBeAg in serum reflects continuing viral replication and correlates with serum DNA-polymerase levels.<sup>7</sup> In contrast, although sera from patients with fulminant hepatic failure have been shown to contain DNA polymerase<sup>7</sup> HBeAg was present in only two of 17 such patients in the present series despite the use of a highly sensitive solid-phase assay. Thus the early appearance of both anti-HBs and anti-HBe in some of the patients with fulminant hepatic failure indicates that the rapid clearance of their respective antigens may in part be because of enhanced antibody production. In addition, increased humoral responses to HBcAg were also observed with significantly greater anti-HBc IgM concentrations in the fulminant hepatic failure patients, indicating that the enhanced antibody responses are directed at all three HBV antigens. It is of interest that the one patient with uncomplicated acute hepatitis B who had a high concentration of IgM anti-HBc, although never encephalopathic, had moderately severe hepatitis as judged by the clinical course and transaminase concentrations. The brisk responses to the HBV antigens shown here may have important implications for the pathogenesis of

fulminant hepatic failure. As the spleen is the major site of antibody production,<sup>8</sup> the high antibody concentrations in portal blood may combine with HBV antigens released from hepatocytes, resulting in the formation of immune complexes in liver sinusoids.<sup>2</sup> Animal models have shown hepatic necrosis after injection of immune complexes into the mesenteric vein. Sabesin reported severe hepatic necrosis with blockage of sinusoids and hepatic microcirculation by 'immune thrombi',<sup>9</sup> the observed ultrastructural changes resembling anoxic hepatic necrosis. The histological changes seen during fulminant hepatic failure ranging from destruction of Rappaport zone 3 to necrosis of entire liver lobules and the absence of a marked inflammatory response are consistent with necrosis after an interruption of the hepatic microcirculation.<sup>10</sup> Immune complexes have been shown in fulminant hepatic failure<sup>11</sup> and their intrahepatic formation, enhanced by the early antibody response in patients with fulminant hepatic failure, may lead to a lesion analogous to the Arthus reaction with ensuing ischaemic necrosis of hepatocytes.

The cause of this heightened antibody production is unclear. As three immunologically distinct antigens are involved it is unlikely to be due to an immune response gene, which, as currently understood, controls the response to a single antigen. Rather, the response is likely to be part of a generalised increase in immune responsiveness to many antigens, including those specific to HBV.

The rapid disappearance of HBsAg in patients with fulminant hepatic failure may make the diagnosis of an acute HBV infection difficult. Both anti-HBs and anti-HBc may be detected for months or longer after acute viral hepatitis<sup>12</sup> and their presence in the serum of a patient with acute liver failure cannot be taken as definitive evidence of acute HBV infection. Two patterns of serological response were observed in these patients. More commonly, HBsAg in low titre with high titre IgM anti-HBc are present often with undetectable HBeAg. Less commonly, HBsAg is not detected in these patients although anti-HBs and total anti-HBc may be detected; the IgM component of anti-HBc must be tested if the diagnosis of acute HBV infection is to be confirmed.

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#### References

- 1 Trepo CG, Robert D, Motin J, Trepo D, Sepetuan M, Prince AM. Hepatitis B antigen (HBsAg) and/or antibodies (Anti-HBs anti-HBc) in fulminant hepatitis: pathogenetic significance and prognostic significance. *Gut* 1976; **17**: 10-18.
- 2 Woolfe IL, Sheik N El, Cullens H *et al.* Enhanced anti-HBs production in the pathogenesis of fulminant viral hepatitis. *Br Med J* 1976; **2**: 669-71.
- 3 Trey G, Davidson CJ. Management of fulminant hepatic failure. In: Popper H, Schaffner F, eds. *Progress in liver diseases*, vol 3. New York: Grune and Stratton, 1970: 282-98.
- 4 Cameron CH, Combridge BS, Howell DR, Barbara JAJ. A sensitive immunoradiometric assay for the detection of hepatitis B surface antigen. *J Virol Meth* 1980; **1**: 311-22.
- 5 Barbara JAJ, Mijovic V, Cleghorn TE, Tedder RS, Briggs M. Liver enzyme concentration as a measure of possible infectivity in chronic asymptomatic carriers of hepatitis B. *Br Med J* 1978; **2**: 1600-02.
- 6 Tedder RS, Wilson Croome R. Detection by radioimmunoassay of IgM class antibody to hepatitis B core antigen: comparison of two methods. *J Med Virol* 1980; **6**: 235-47.
- 7 Alberti A, Diana J, Eddleston ALWF, Williams R. Changes in acute hepatitis B virus DNA polymerase in relation to outcome of hepatitis B. *Gut* 1979; **20**: 190-5.
- 8 Rowley DA. Formation of circulating antibody in splenectomised humans following IV injection of heterologous erythrocytes. *J Immunol* 1950; **65**: 515-22.
- 9 Sabesin JM. Electron-microscopy of hypersensitivity reactions: allergic hepatic necrosis. *Am J Pathol* 1963; **42**: 743-52.
- 10 Horney JT, Galambos JT. The liver during and after fulminant hepatitis. *Gastroenterology* 1977; **73**: 639-45.
- 11 Canalese J, Wyke RJ, Vergani D, Eddleston ALWF, Williams R. Circulating immune complexes in patients with fulminant hepatic failure. *Gut* 1981; **22**: 845-8.
- 12 Hoofnagle JH, Seef LB, Bales ZB, Gerety RJ, Tabor E. Serologic responses in hepatitis B. In: *Viral hepatitis*. Mas GN, Cohen SW, eds. Philadelphia: Abacus Press, 1978: 219-42.