(3) Doctors interested in asthma should organise an Edinburgh-type admission service for selected patients from their clinics.

(4) Patients attending the asthma clinic should be encouraged to refer themselves back if their condition is deteriorating and should be prepared to put themselves on high-dose steroids at the same time.

(5) Every doctor looking after patients with asthma should be aware of the need for objective measurements (pulse rate and peak flow rate) in assessing the severity of the disease in his patients and should know how to institute emergency treatment. In severe cases the doctor should accompany the patient to hospital. Discharge from hospital of recovering patients should occur when objective measurements show a return to the patient's normal values. Steroid treatment should be continued at least until the patient is seen in the clinic.

We are most grateful to Dr Elspeth Macdonald, Mrs Daphne Thomas, and the consultant physicians and general practitioners of the area for their help in the study.

References

- 1 Speizer, F E, Doll, R, and Heaf, P, *British Medical Journal*, 1968, **1**, 335.
- 2 Speizer, F E, *et al*, *British Medical Journal*, 1968, **1**, 339.
- 3 Inman, W H W, and Adelstein, A M, *Lancet*, 1969, **2**, 279.
- 4 Stolley, P D, *American Review of Respiratory Diseases*, 1972, **105**, 883.
- 5 Gandevia, B, *Medical Journal of Australia*, 1968, **1**, 884.
- 6 Herxheimer, H, *American Review of Respiratory Diseases*, 1973, **107**, 306.
- 7 Gandevia, B, *Medical Journal of Australia*, 1973, **1**, 273.
- 8 Burr, M L, *et al*, *Thorax*, 1975, **30**, 663.
- 9 Burr, M L, personal communication.
- 10 Frazer, P M, *et al*, *British Journal of Diseases of the Chest*, 1971, **65**, 71.
- 11 Crompton, G K, and Grant, I W B, *British Medical Journal*, 1975, **4**, 680.
- 12 Rebuck, A S, and Read, J, *American Journal of Medicine*, 1971, **51**, 788.
- 13 Williams, S J, and Seaton, A, submitted for publication.

Immune response to HBsAg and the spectrum of liver lesions in HBsAg-positive patients with chronic renal disease

R M GALBRAITH, N EL SHEIKH, B PORTMANN, A L W F EDDLESTON,
ROGER WILLIAMS, V PARSONS, M BEWICK, C S OGG

British Medical Journal, 1976, **1**, 1495-1497

Summary

Evidence of chronic hepatitis was found on histological examination in nine out of 15 patients positive for hepatitis-B surface antigen (HBsAg) who had either chronic renal failure or a functioning renal transplant. Cirrhosis had already developed in three of the patients, who deteriorated rapidly and died. Liver biopsies from the remaining 12 patients showed the features of chronic aggressive hepatitis in two, chronic persistent hepatitis in four, and minor histological lesions in six. The persistence of HBsAg in patients with renal failure or in those receiving immunosuppressive drugs after a transplant must indicate some impairment of the normal immune response to hepatitis-B viral antigens. Nevertheless, cellular or humoral immunity to HBsAg was detected in all eight patients with chronic hepatitis tested compared with only one out of five with minimal liver lesions, which suggests that the severity of the liver damage may be directly related to the degree of immunocompetence.

Introduction

Infection with the hepatitis-B virus has been common in both haemodialysis and renal transplantation units.¹ Although some patients develop typical acute hepatitis, the course in most is more insidious. Biochemical abnormalities of liver function are less pronounced, hepatitis-B surface antigenaemia often persists, and progression to chronic liver disease may occur.² We report here a systematic investigation of liver dysfunction in a consecutive series of 15 such patients who had had hepatitis-B surface antigens (HBsAg) for six months or longer. Since immunosuppressive treatment may modify the underlying hepatic lesion, we compared the immune responses to HBsAg and the associated histological changes in patients receiving immunosuppressive treatment after successful renal transplantation with those responses demonstrable in patients maintained on haemodialysis or by dietary protein restriction alone.

Patients and methods

Of the 15 patients with chronic renal failure, two had been managed by protein restriction alone and seven were maintained on intermittent haemodialysis. The other six had functioning renal transplants and were receiving immunosuppressive treatment consisting of prednisolone and azathioprine. Tests for HBsAg including immunodiffusion, complement fixation, and radioimmunoassay³ gave positive results in every case. Four of the patients undergoing haemodialysis and all the transplant recipients had become HBsAg-positive during an outbreak of hepatitis-B infection in the renal unit at Guy's Hospital in 1969-1971⁴ and remained positive since. The remaining five patients were referred from other hospitals and had been HBsAg-positive for six to 18 months.

In addition to clinical examination and standard liver function tests (see table), a liver biopsy was carried out in each case for histological examination. Paraffin sections were also examined for HBsAg using both specific immunofluorescence and the orcein stain.⁵

Liver Unit, King's College Hospital and Medical School, Denmark Hill, London SE5

R M GALBRAITH, MB, MRCP, lecturer in medicine

N EL SHEIKH, MB, BS, visiting research fellow

B PORTMANN, MD, research fellow

A L W F EDDLESTON, DM, MRCP, senior lecturer and consultant physician

ROGER WILLIAMS, MD, FRCP, director of unit and consultant physician

V PARSONS, DM, FRCP, renal physician

Department of Nephrology, Guy's Hospital, London SE1

M BEWICK, FRCS, senior lecturer in surgery

C S OGG, MD, FRCP, consultant renal physician

Clinical findings and other data in the 15 patients

Case No	Sex	Age (years)	Renal disease*	Liver (cm palpable below costal margin)	spleen	Bilirubin ($\mu\text{mol/l}$) (normal <20)	Aspartate aminotransferase (IU/l) (normal ≤ 50)	Alkaline phosphatase (IU/l) (normal <85)	Prothrombin time (s prolonged)	Albumin (g/l) (normal >35)	Leucocyte migration index (HBsAg) (normal ≥ 0.8)	Dane particles	HBsAg complexes	Liver histology†
<i>Patients on haemodialysis</i>														
1	M	44	GN	0	4	47	234	138	5-13	14	ND	ND	ND	CAH, cirrhosis
2	F	32	TB	4	2	Normal	83	188	0	29	0.93	+	+	CAH, granulomas
3	M	24	GN	0	0	"	57	Normal	0	Normal	0.78	+	+	CPH
4	F	29	PN	4	0	"	113	"	0	"	0.70	+	-	CPH, siderosis
5	M	28	GN	0	0	"	Normal	194	0	"	0.84	-	-	FPN
6	M	30	GN	0	0	"	"	150	0	"	0.86	-	-	FPN, siderosis
7	M	33	GN	0	0	"	98	208	0	"	0.81	-	-	FPN, siderosis
<i>Patients with transplants</i>														
8	M	35	PN	2	4	66	800	126	13	23	ND	+	+	CAH, cirrhosis
9	F	31	GN	0	0	Normal	Normal	Normal	0	Normal	0.39	+	++	CAH
10	F	25	ECL	0	0	"	255	"	0	"	0.53	+	++	CPH
11	F	22	PN	0	0	"	Normal	"	0	"	0.43	+	++	CPH
12	M	18	PN	0	0	"	"	113	0	"	0.51	+	++	N
13	M	50	PCD	3	0	"	75	Normal	0	"	0.94	-	-	N
<i>Patients on diet alone</i>														
14	M	53	GN	0	0	35	172	133	18-13	29	ND	++	++	CAH, cirrhosis
15	M	29	GN	2	0	Normal	Normal	Normal	0	Normal	ND	ND	ND	FPN

*GN = Glomerulonephritis. TB = Tuberculosis. PN = Pyelonephritis. ECL = Eclamptic toxæmia. PCD = Polycystic disease.

†CAH = Chronic aggressive hepatitis. CPH = Chronic persistent hepatitis. FPN = Focal parenchymal necrosis. N = Minimal changes. ND = Not done.

Cellular immunity to HBsAg was investigated using the leucocyte migration test with partially purified HBsAg as antigen. Migration indices of less than 0.8 indicated a positive response.⁶ Since all patients were positive for HBsAg the presence of antibody was inferred when serum examined by electron microscope showed antigen complexes. Serum from each patient was also screened by immunodiffusion for e-antigen, which is an accurate marker of chronic hepatitis in HBsAg-positive patients.^{7, 8}

Results

Three patients (cases 1, 8, and 14) were jaundiced; one (case 8) also had spider naevi and hepatosplenomegaly, and splenomegaly was present in case 14. Two patients (cases 1 and 14) had developed ascites within the previous month. In the remaining 12 patients clinical evidence of liver dysfunction was less obvious. None were jaundiced or had cutaneous stigmata of chronic hepatic disease. In four, however, the liver was enlarged on examination (cases 2, 4, 13, and 15), and in case 2 the spleen was also palpable.

Liver function tests in the three patients with clinical jaundice (cases 1, 8, and 14) showed that serum aspartate aminotransferase (SGOT) values were raised as well as serum bilirubin levels. Serum albumin levels were reduced and prothrombin times were prolonged in all three. Raised serum alkaline phosphatase levels in all three patients were shown by electrophoresis to be predominantly in the hepatic fraction. In six of the other 12 patients SGOT levels were also raised. These included three of the four patients with hepatomegaly. Alkaline phosphatase levels were raised in five. These increases were mainly of bony origin, except in the patient whose albumin level was slightly reduced (case 2).

Histological examination of the liver biopsy specimens showed a spectrum of lesions (see table). The changes in cases 1, 8, and 14 were characteristic of chronic aggressive hepatitis with established cirrhosis. Two other patients (cases 2 and 9) had chronic aggressive hepatitis but without the development of cirrhosis. In cases 3, 4, 10, and 11, the histological diagnosis was chronic persistent hepatitis. Thus chronic hepatitis was present in no fewer than nine (60%) of the 15 patients. The remaining six patients (cases 5, 6, 7, 12, 13, and 15) had either normal appearances, apart from the presence of HBsAg in some hepatocytes, or occasional well-defined areas of single cell necrosis in the parenchyma, a picture termed by other workers "focal parenchymal necrosis."⁹

There was no significant differences in the incidence of the various histological lesions between patients maintained on haemodialysis and transplant recipients. Two patients from each group showed the changes of chronic aggressive hepatitis and two patients had chronic persistent hepatitis. Cirrhosis had developed in one patient from each group. Of the two patients managed by dietary protein restriction alone, one had chronic aggressive hepatitis with cirrhosis and the other focal parenchymal necrosis. Only three patients on haemodialysis who had received regular parenteral injections of iron showed gross iron deposits on liver biopsy (grade 4 siderosis).¹⁰

CLINICAL FOLLOW-UP

All three patients with clinical jaundice and chronic aggressive hepatitis (cases 1, 8, and 14) deteriorated rapidly and died. One of these patients (case 8), who had been taking prednisolone and azathioprine after a renal transplant carried out five years previously, had apparently developed this rapidly progressive disease despite immunosuppressive treatment. Azathioprine was withdrawn at the time of the present admission because of potential hepatotoxicity and the dose of prednisolone was considerably increased, but despite this his liver function deteriorated inexorably and he died two weeks later. Another (case 14) had been on corticosteroids for the preceding six months, but his condition deteriorated rapidly after admission and he also died a few weeks later. The third patient (case 1), who had not been given immunosuppressive treatment, died within 48 hours of admission for further investigations from spontaneous peritonitis, a well-recognised complication of cirrhosis.

The remaining two patients with chronic aggressive hepatitis were still alive at the time of writing. The patient with a transplant (case 9) was receiving corticosteroids and azathioprine and had no clinical abnormalities. The other patient (case 2), whose liver biopsy showed numerous granulomata, was given a six-month course of rifampicin, para-aminosalicylic acid, and isoniazid. There was no change in the non-caseating granuloma on follow-up liver biopsy, however, and she then started prednisolone and azathioprine treatment with improvement both symptomatically and in the liver function test results. We have recently observed similar granulomatous lesions, which do not contain acid-fast bacilli, in other patients on haemodialysis and have suggested that they may represent a tissue reaction in an immunosuppressed patient to organisms that are normally non-pathogenic or to emboli of biologically inert foreign material entering the circulation during dialysis.¹¹

IMMUNOLOGICAL STUDIES

Inhibition of leucocyte migration using HBsAg as antigen was detected in five of the six patients with chronic hepatitis who were examined. Complexes of HBsAg were seen on electronmicroscopy in the sera of five of these patients and in two further patients who were not tested for cellular immunity. Thus evidence of cellular or humoral immunity to HBsAg, or both, was detected in all eight patients with chronic hepatitis who were tested. In contrast such evidence of immunity was observed in only one of the five patients with minimal liver lesions tested, and this difference was statistically significant ($\chi^2 = 5.8$; $P < 0.025$). The complexes consisted of 22-nm spherical and tubular forms of the antigen, but larger 42-nm Dane particles were also often present. The latter were also found in the one patient with chronic hepatitis who did not have complexes in the serum (case 4).

Hepatocytes containing HBsAg in the cytoplasm were shown, both by specific immunofluorescence and by orcein staining, in seven of the nine patients with chronic hepatitis, but positive cells were few ($< 1\%$) and distributed in a spotty random fashion throughout the lobule. In contrast, the biopsy specimens from all five patients with minimal liver lesions contained many positive hepatocytes arrayed in sheets with intervening negative zones. These were also seen as typical "ground glass" cells in haematoxylin and eosin preparations.⁵

Tests for the e-antigen/antibody showed that three patients, two with chronic aggressive hepatitis (cases 1 and 14) and one with chronic persistent hepatitis (case 3), had the antigen. None of the patients were positive for the antibody. Abnormal immunoglobulin levels were detected in only one patient (case 9), who had a moderate increase in both IgG and IgM levels.

Discussion

The development of chronic hepatitis in our patients is probably related to impairment of the normal immune response to hepatitis B viral antigens. As a result, infected hepatocytes may be destroyed but the virus is never completely eliminated.¹²⁻¹³ If the defect is complete liver damage may be minimal or even absent, as in the healthy carrier state. In support of this, Lee *et al* have shown that the frequency of cellular immunity is much higher in HBsAg-positive blood donors with chronic hepatitis than in those who are healthy carriers of the antigen.¹⁴ Our findings are further evidence for this hypothesis, since all eight patients with chronic hepatitis who were tested showed either cellular or humoral immunity to HBsAg compared with only one out of five with minor liver lesions. Furthermore, as has been shown,^{3, 15} there were fewer hepatocytes containing HBsAg in liver biopsy specimens from patients with chronic hepatitis than in those from patients with minimal histological abnormalities only.

A comparison of the spectrum of histological lesions in our patients with that reported in 38 HBsAg-positive blood donors of similar ethnic origin but without renal disease⁹ shows that the incidence of chronic aggressive hepatitis appears to be higher among our patients (33% as compared with 12%). This may be related to the finding that cellular immunity to HBsAg was commoner in our patients than in those blood donors who were tested immunologically.¹⁴ The underlying cause of the impaired immune response to hepatitis B viral antigens is likely to be of prime importance. In blood donors the defect is thought to be genetically determined¹⁶ and is probably specific¹¹ since other measurements of cellular immunity such as the phytohaemagglutinin response are often normal.¹⁷

In contrast, in patients with renal disease the defect is more likely to reflect only one facet of a generalised and non-specific depression of immunity, due either to a toxic effect of renal failure in patients maintained by haemodialysis or protein restriction alone¹⁸ or to the immunosuppressive treatment given after a transplant. Possibly the effect of antirejection treatment is relatively more immunosuppressive than that of renal failure, since after the original outbreak at Guy's Hospital¹ none of the 12 transplant recipients treated with prednisolone or azathioprine ever reverted to being HBsAg-negative, whereas antigen clearance occurred in three of 10 patients who were

treated by dialysis alone and survived without transplantation for at least six months after their routine test for HBsAg showed them to be positive. This is also the experience of at least one other unit.¹⁹ Nevertheless, in our series no appreciable differences in immune response to HBsAg, or indeed in the proportion with chronic hepatitis, were apparent between patients on haemodialysis and those with functioning transplants.

Three patients with renal disease and chronic aggressive hepatitis have already died in hepatocellular failure and it would seem reasonable to treat similar cases with immunosuppressive drugs to try to improve the prognosis. The patients with chronic persistent hepatitis will require careful follow-up since evolution to an aggressive lesion requiring immunosuppressive treatment has occurred in at least one other patient with renal disease.¹¹ Furthermore, in HBsAg-positive patients the presence of e-antigen may be associated with a particularly high risk of such progression.⁸ In patients with successful transplants much debate has centred on whether azathioprine given to prevent rejection can itself provoke chronic liver damage.²⁰ The incidence of chronic liver damage, however, was no greater in our transplant recipients than in patients remaining on dialysis, and this possibility has now been largely discounted by several studies in which withdrawal of azathioprine led, as in case 8, to no appreciable clinical improvement.^{21, 22}

An alternative approach to the treatment of HBsAg-positive chronic liver disease in renal recipients would be to reduce all immunosuppressive treatment to a minimum, or even withdraw it completely, since this may allow a return of immunocompetence and the development of acute hepatitis with subsequent elimination of the virus. This has been reported in one patient,²³ but, apart from the risks of rejection of the graft, such an approach would be potentially hazardous if there were many infected cells.²⁴ In such patients re-establishing normal immune responses could result in massive hepatic necrosis with death in fulminant hepatic failure, as we have reported in three persistently HBsAg-positive patients with malignant disease in whom cytotoxic treatment was withdrawn.²⁵

We are grateful to the department of chemical pathology, King's College Hospital, for liver function tests, to Ms Helen Cullens and Professor A J Zuckermann for HBsAg testing, and to the Wellcome Trust for support.

References

- Public Health Laboratory Service, *British Medical Journal*, 1974, **4**, 751.
- Ware, A J, *et al*, *Gastroenterology*, 1975, **68**, 755.
- Reed, W D, *et al*, *Lancet*, 1973, **2**, 690.
- Ogg, C S, *et al*, *Proceedings of the European Dialysis and Transplantation Association*, 1972, **9**, 228.
- Portmann, B, *et al*, *Gut*, 1976, **17**, 1.
- Reed, W D, *et al*, *Lancet*, 1974, **1**, 581.
- Magnius, L O, *et al*, *Journal of the American Medical Association*, 1975, **231**, 356.
- El Sheikh, N, *et al*, *British Medical Journal*, 1975, **4**, 252.
- Woolf, I L, *et al*, *Journal of Clinical Pathology*, 1974, **27**, 348.
- Scheuer, P J, Williams, R, and Muir, A R, *Journal of Pathology and Bacteriology*, 1962, **84**, 53.
- Galbraith, R M, *et al*, *Lancet*, 1975, **2**, 886.
- Dudley, F J, Fox, R A, and Sherlock, S, *Lancet*, 1972, **1**, 723.
- Eddleston, A L W F, and Williams, R, *Lancet*, 1974, **2**, 1543.
- Lee, W M, *et al*, *Gut*, 1975, **16**, 416.
- Deodhar, K P, Tapp, E, and Scheuer, P J, *Journal of Clinical Pathology*, 1975, **28**, 66.
- Blumberg, B S, *et al*, *Proceedings of the National Academy of Science*, 1969, **62**, 1108.
- Nielsen, J O, *et al*, *Clinical and Experimental Immunology*, 1973, **15**, 9.
- Newberry, W M, and Sanford, J P, *Journal of Clinical Investigation*, 1971, **50**, 1262.
- Zazgornik, J, *et al*, *Medicine and Chirurgie Digestives*, 1975, **4**, 81.
- Zarday, Z, *et al*, *Journal of the American Medical Association*, 1972, **222**, 690.
- Ireland, P, *et al*, *Archives of Internal Medicine*, 1973, **132**, 29.
- Briggs, W A, *et al*, *Archives of Internal Medicine*, 1973, **132**, 21.
- Reed, W, *et al*, *Transplantation Proceedings*, 1971, **3**, 343.
- Gudat, F, *et al*, *Laboratory Investigation*, 1975, **32**, 1.
- Galbraith, R M, *et al*, *Lancet*, 1975, **2**, 528.