

# Significance of delta agent infection in chronic hepatitis B virus infection: a study in British carriers

I V D WELLER, P KARAYIANNIS, A S F LOK, L MONTANO,  
M BAMBER, H C THOMAS, AND SHEILA SHERLOCK

*From the Department of Medicine, Royal Free Hospital and School of Medicine, London*

**SUMMARY** Delta antigen ( $\delta$ ) is a transmissible agent requiring hepatitis B virus (HBV) for its replication. Antibody to  $\delta$  (anti- $\delta$ ) was present in nine of 71 (13%) British HBV carriers: six were intravenous drug abusers and two were haemophiliacs. Anti- $\delta$  was negative in 30 HBsAg positive homosexuals. Cirrhosis was common in patients with anti- $\delta$  and those with anti- $\delta$  positive cirrhosis were significantly younger than those with anti- $\delta$  negative cirrhosis. In British HBV carriers  $\delta$  infection is associated with intravenous drug abuse and haemophilia and perhaps a more rapid progression of chronic liver disease.

Delta antigen ( $\delta$ ) was discovered by direct immunofluorescence in hepatocyte nuclei of Italian patients with chronic hepatitis B virus (HBV) infection; it is distinct from other HBV antigens.<sup>1</sup> Hepatitis B virus carriers with  $\delta$  in the liver have a serum antibody (anti- $\delta$ ) in high titre detectable by radioimmunoassay and  $\delta$  itself has been extracted from liver and serum.<sup>2-4</sup> Delta antigen from liver is a protein of 68 000 daltons.<sup>2</sup> In serum it is associated with a low molecular weight RNA encapsulated by hepatitis B surface antigen (HBsAg) in a 35-37 nm subpopulation of particles.<sup>3, 4</sup> Studies in chimpanzees suggest that  $\delta$  is a marker of a transmissible pathogenic agent, either a variant of HBV or another virus which requires HBV for its replication.<sup>5, 6</sup> Anti- $\delta$  is prevalent in Italian HBV carriers and more common in patients with chronic liver disease.<sup>7</sup> The prevalence of anti- $\delta$  in British HBV carriers is unknown. The aim of this study was to determine whether  $\delta$  infection is common in British HBV carriers and its significance in terms of predicting the severity of chronic liver disease.

## Methods

### PATIENTS

Seventy one HBsAg carriers born and resident in Great Britain were studied. Sixty four men and seven women; mean age 36 years (range 11-67 years). Forty five were HBeAg and 26 anti-HBe

positive. Fifty nine patients had abnormal liver function tests and 50 histologically proven chronic liver disease (nine chronic persistent and 28 chronic active hepatitis, eight active and five inactive cirrhosis). The patients included 30 male homosexuals, 10 intravenous drug abusers, and three haemophiliacs. Serum taken at the time of liver biopsy and in 16 patients multiple samples taken over one to four years were stored at  $-20^{\circ}\text{C}$ . Serum was also collected from 100 British blood donors, 79 British haemophiliacs and 58 British intravenous drug abusers, all were HBsAg negative.

HBsAg, HBeAg, anti-HBe, anti-HBc, and anti-HBs were detected by radioimmunoassay (Abbott). Sera were coded and sent for 'blind' analysis for anti- $\delta$  to Dr Mario Rizzetto (Turin, Italy). Anti- $\delta$  was detected and titred by a solid phase blocking radioimmunoassay<sup>2</sup> (titres  $>1:10^3$  indicate current infection and  $<1:10^3$  past infection, with  $\delta$ ).

Liver biopsies from six anti- $\delta$  positive and 10 anti- $\delta$  negative carriers were embedded in OCT and snap frozen. Five microns thick cryostat sections were fixed in acetone/chloroform, lyophilised for two hours and then stained for  $\delta$  antigen by direct immunofluorescence with a fluorescein-isothiocyanate (FITC) labelled antiserum to  $\delta$  (donated by Dr M Rizzetto). The preparation and specificity of this antiserum has been described.<sup>1, 8</sup>

## Results

### HBsAg POSITIVE CARRIERS

Anti- $\delta$  was positive in nine of 71 carriers (13%)

Address for correspondence: Dr H C Thomas, The Royal Free Hospital, Pond Street, London NW3 2QG.

Received for publication 28 January 1983

(Table 1). In the carriers in whom multiple serum samples were analysed over several years, three were anti- $\delta$  positive and 13 anti- $\delta$  negative on all specimens. Of the nine anti- $\delta$  positive carriers four were HBeAg and five anti-HBe positive, six were intravenous drug abusers, and two were haemophiliacs. The ninth was a 52 year old heterosexual male with cirrhosis and primary hepatocellular carcinoma who had never abused drugs. Eight patients had anti- $\delta$  titres of  $>1$  in  $10^3$  and one a titre of  $<1$  in  $10^3$ . Delta antigen was detected in all six liver biopsies from these anti- $\delta$  positive carriers and in none of the 10 anti- $\delta$  negative carriers (Figure).

None of the 30 homosexual males was anti- $\delta$  positive. The prevalence of anti- $\delta$  in the non-haemophiliacs, non-intravenous drug abuser carriers was one of 58 (2%).

Cirrhosis was common in anti- $\delta$  positive carriers that had liver biopsies; four of seven compared with nine of 46 in the anti- $\delta$  negative carriers (Table 2). The four with anti- $\delta$  positive cirrhosis were significantly younger (30, 31, 30, and 52 years) than the nine with anti- $\delta$  negative cirrhosis (median 56 years, range 33–67 years,  $p < 0.05$ , Wilcoxon's rank sum test). The 31 year old carrier with anti- $\delta$  positive cirrhosis died recently, six months after the biopsy, with end stage liver disease. Of the other five anti- $\delta$  positive patients three had a chronic active hepatitis and two had raised transaminases but no biopsy was taken (one was a haemophiliac and the other a drug addict who refused). None of the carriers with normal liver function tests and/or minimal change

Table 1 Prevalence of anti- $\delta$  in patient groups

Patient group	No	Anti- $\delta$ positive	%
<b>HBsAg-positive carriers</b>			
Male homosexuals	30	0	
Intravenous drug abusers	10	6	
Haemophiliacs	3	2	
Others	28	1	
Total	71	9*	13
<b>HBsAg-negative controls</b>			
Blood donors	100	0	0
Haemophiliacs	79	0	0
Intravenous drug abusers	58	2†	3

\* Titres  $>1$  in  $10^3$ .

† Titres  $<1$  in  $10^3$ .

on biopsy (12) or chronic persistent hepatitis (nine) had anti- $\delta$ .

#### HBsAg NEGATIVE BLOOD DONORS, HAEMOPHILIACS, AND DRUG ADDICTS

None of the 100 HBsAg negative blood donors and 79 haemophiliacs was anti- $\delta$  positive although 46 of the haemophiliacs had anti-HBs and/or anti-HBc. Two of the 58 HBsAg negative intravenous drug abusers had anti- $\delta$  in low titre and both had anti-HBs and anti-HBc as did 39 of the other abusers.

#### Discussion

Nine out of 71 (13%) of British HBsAg carriers

Figure Delta antigen staining in hepatocyte nuclei of an anti- $\delta$  positive drug abuser: direct immunofluorescence with anti- $\delta$ -FITC antiserum ( $\times 346$ ).

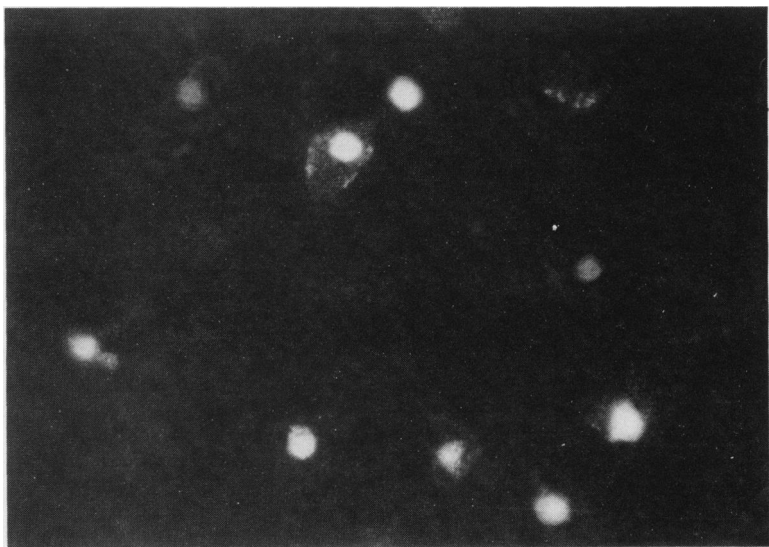


Table 2 HBsAg-positive carriers: liver histology in relation to  $\delta$  infection

Histology	Normal LFTs no biopsy or min change	Abnormal LFTs				
		No biopsy	CPH	CAH	AC	IC
Anti- $\delta$ -ve	12	7	9	25	5	4
Anti- $\delta$ +ve	0	2	0	3	3	1

CPH = chronic persistent hepatitis, CAH = chronic active hepatitis, IC = inactive cirrhosis, AC = active cirrhosis, LFTs = liver function tests.

were anti- $\delta$  positive. The prevalence of anti- $\delta$  is high in Northern and Southern Italy (16–29% and 59% respectively) and low in Germany, Poland, and France (1–9%). A very high prevalence was found in Scandinavia and two American states (23–47%), but these populations contained a high proportion of intravenous drugs abusers and/or multiply-transfused subjects,<sup>7</sup> who are more exposed to repeated HBV and therefore  $\delta$  infection.<sup>9,10</sup> In our study eight of nine anti- $\delta$  positive patients were either intravenous drug abusers or haemophiliacs. Excluding these the prevalence is one out of 58 (2%), more in keeping with other European studies. Two HBsAg negative drug abusers with evidence of past HBV infection had anti- $\delta$  in low titre and this is thought to be owing to a past infection with both hepatitis B virus and  $\delta$ .<sup>9</sup>

All 30 homosexual men were anti- $\delta$  negative. This group, like intravenous drug abusers and haemophiliacs has a high incidence of HBV infection, and therefore might be expected to develop  $\delta$  infection. This may be related to a lower inoculum of virus with sexual transmission allowing HBV but not  $\delta$  infection. In the chimpanzee a  $10^{-8}$  dilution of HBV/ $\delta$  containing serum has resulted in loss of  $\delta$  but retention of HBV infectivity.<sup>3</sup> An alternative possibility is that delta infection has not yet been introduced to a great extent into the homosexual population.

Cirrhosis was more common in patients with anti- $\delta$  than in those without and patients with anti- $\delta$  positive cirrhosis were significantly younger than those with anti- $\delta$  negative cirrhosis. This suggests that the presence of delta agent may be associated with a more rapid progression of chronic liver disease. Other factors associated with intravenous drug abuse and haemophilia, such as concomitant NANB virus infection,<sup>11,12</sup> and an early age of initial HBV infection, may also play a role.

We thank Dr M Rizzetto for performing the anti- $\delta$  assay. IVDW was an MRC Training Fellow and is

now lecturer in the Academic Department of Genitourinary Medicine, Middlesex Hospital. PK and MB were also supported by the MRC. ASFC and LM were supported by the University of Hong Kong and CONACYT Mexico respectively. HCT is a Senior Wellcome Fellow. This work was presented in part at the autumn meeting of the British Society of Gastroenterology, Leeds, 1982.

## References

- Rizzetto M, Canese MG, Arico S, Crivelli O, Bonino F, Trepo C, Verme G. Immunofluorescence detection of a new antigen-antibody system ( $\delta$ /anti- $\delta$ ) associated with the hepatitis B virus in the liver and in the serum of HBsAg carriers. *Gut* 1977; **18**: 997–1003.
- Rizzetto M, Shih JW-K, Gerin JL. The hepatitis B virus-associated  $\delta$  antigen: isolation from liver, development of solid-phase radioimmunoassays for  $\delta$  antigen and anti- $\delta$  and partial characterisation of  $\delta$  antigen. *J Immunol* 1980; **125**: 318–24.
- Rizzetto M, Hoyer B, Canese MG, Shih JW-K, Purcell RH, Gerin JL.  $\delta$  agent: the association of  $\delta$  antigen with hepatitis B surface antigen and RNA in serum of  $\delta$ -infected chimpanzees. *Proc Natl Acad Sci USA* 1980; **77**: 6124–8.
- Bonino F, Hoyer B, Ford E, Shih JW-K, Purcell RH, Gerin JL. The  $\delta$ -agent: HBsAg particles with  $\delta$  antigen and RNA in the serum of an HBV carrier. *Hepatology* 1981; **1**: 127–31.
- Rizzetto M, Canese MG, Gerin JL, London WT, Sly DL, Purcell RH. Transmission of the hepatitis B virus-associated delta antigen to chimpanzees. *J Infect Dis* 1980; **141**: 590–602.
- Leading article. Delta agent – a virus in disguise. *Lancet* 1982; **1**: 259–60.
- Rizzetto M, Purcell RH, Gerin JL. Epidemiology of HBV-associated delta antigen. Geographical distribution and prevalence in poly-transfused HBsAg carriers. *Lancet* 1980; **1**: 1215–8.
- Stoeklin E, Gudat F, Krey G *et al.*  $\delta$  antigen in hepatitis B: immunohistology of frozen and paraffin-embedded liver biopsies and relation to HBV-infection. *Hepatology* 1981; **1**: 238–42.
- Raimondo G, Smedile A, Gallo L, Balbo A, Panzetto A, Rizzetto M. Multicentre study of prevalence of HBV-associated delta infection and liver disease in drug addicts. *Lancet* 1982; **1**: 249–51.
- Rizzetto M, Morello C, Mannucci PM *et al.* Delta infection and liver disease in haemophiliac carriers of hepatitis B surface antigen. *J Infect Dis* 1982; **145**: 18–22.
- Knodel RG, Conrad ME, Ishak KG. Development of chronic liver disease after acute non-A, non-B post transfusion hepatitis. *Gastroenterology* 1977; **72**: 902–9.
- Bamber M, Murray A, Arborgh BAM, Scheuer PJ, Kernoff PBA, Thomas HC, Sherlock S. Short incubation non-A, non-B hepatitis transmitted by factor VIII concentrates in patients with congenital coagulation disorders. *Gut* 1981; **22**: 854–9.