Clin. exp. Immunol. (1985) **61**, 39–43.

Absence of autoimmune serological reactions in chronic non A, non B viral hepatitis

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(Accepted for publication 28 January 1985)

SUMMARY

In 18 cases of chronic liver disease due to non-A, non-B hepatitis virus(es) in which the diagnosis was established by transmission, including chimpanzee inoculation in nine, sera were tested for the autoantibodies characteristically associated with autoimmune chronic active hepatitis. The frequency of autoantibodies to nuclear, smooth muscle, cytofilament, mitochondrial and liver membrane antigens was low, being not greater than that recorded for a normal population, and the few positive reactions obtained were at very low titre. These findings suggest that among cases of 'HBsAg negative' chronic hepatitis, those due to NANB infection are distinguishable from those due to autoimmune chronic hepatitis by negative serological tests for autoantibodies.

Keywords post-transfusion hepatitis non A, non B hepatitis autoantibodies liver antigens

INTRODUCTION

The initially described form of chronic active hepatitis (CAH), which gave rise to the classic descriptions, was that associated with marked periportal necrosis and cellular infiltration in the liver and serological abnormalities in the blood; it was described as lupoid hepatitis and, subsequently, as autoimmune CAH (Mackay, 1975). However CAH is now known to comprise a 'spectrum' of diseases including hepatitis B virus infection, drug sensitivity, Wilson's disease, ethanol abuse and α_1-anti-trypsin deficiency (Hodges, Millward-Sadler & Wright, 1982). Unresolved non-A, non-B (NANB) viral hepatitis, whether occurring after blood transfusion or sporadically, is another component of this spectrum and, if as is stated (Robinson, 1982; Dienstag, 1983), some 7–10% of recipients of transfused blood in USA develop NANB post-transfusion hepatitis, and abnormal liver function tests persist for greater than one year in a high proportion of these, with some 30% developing chronic hepatitis, then NANB-CAH could make a substantial contribution to all cases of CAH. In addition, sporadic non-parenterally acquired NANB hepatitis which has a high incidence among recently reported case series of acute infectious hepatitis (Farrow *et al.*, 1981; Frøland, Teien & Ulstrop, 1982) could also contribute cases of NANB-CAH, although the frequency of evolution of sporadic NANB hepatitis to CAH is unknown.

Because of the lack of any known inciting cause, autoimmune CAH could, literally, be regarded as 'non-A, non-B' since, in some 30% of cases, there are no serological markers of past infection with

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either hepatitis A or B viruses (Mackay, 1979); however differentiation of NANB and autoimmune CAH could be important in view of the good response of the latter type to treatment. Hence sera from subjects in whom the diagnosis of NANB chronic hepatitis had been established by transmission of disease, either to man or chimpanzees, were tested for the serological markers characteristic of autoimmune CAH. The NANB sera tested, referred under code by one of the authors (HJA) from the National Institutes of Health, Bethesda, USA, comprise a standard reference panel used hitherto for evaluation of proposed serological tests for the detection of NANB agents in serum.

MATERIALS AND METHODS

Serum panel. The panel of 24 sera referred from the NIH was a pre-coded standard panel originally prepared for the assessment of 'candidate' assays for serological detection of NANB agents in human serum. Details of cases are shown in Table 1. This panel included 18 sera from cases of chronic hepatitis which lacked markers of infection with hepatitis A or B viruses, and accordingly could be attributed to NANB infection with the additional evidence that these sera contained an agent capable of transmitting hepatitis. One serum sample only was tested for each case, this being generally obtained at the time of the initial liver biopsy. The controls used are cited in Results.

Serological procedures for autoantibodies. Sera were tested for antibodies to nuclei (ANA), smooth muscle (ASMA), the cytofilaments actin and intermediate filaments, mitochondria (AMA) and to liver membrane antigens (LMAg). Standard immunofluorescence procedures were used on cryostat sections (Whittingham, 1972); the fluoresceinated sheep anti-human immunoglobulin used was shown to be reactive with IgG, IgM and IgA. Human blood smears were used to detect

Table 1. Clinical data on patients with chronic NANB hepatitis

Case No.	Units blood	Onset	Peak ALT	Fluct. ALT	Symptoms	Liver biopsy	Chimpanzee transmission	Outcome
1	10	10	615	Yes	++++	CAH	nd	Died, multiple myeloma
2	7	8	415	No	–	CAH	nd	Recovered, 1 year
3	17	13	1,530	No	++	nd	nd	Recovered, 20 weeks
4	13	8	1,600	Yes	++++	CAH	+	Recovered, 6 years
5	8	8	1,200	No	+++	CAH	nd	Died, liver failure
6	5	7	505	Yes	++	CAH	nd	Mild enzyme elevations
7	20	8	450	Yes	–	CAH, cirrhosis	nd	Chronic ALT elevation
8	14	7	785	No	–	CAH	±	Chronic ALT elevation
9	7	3	800	Yes	+++	nd	nd	Recovered, 3 years
10	13	12	308	No	–	CAH	nd	Chronic ALT elevation
11	13	4	468	Yes	++	CAH	+	Chronic ALT elevation
12	20	10	918	Yes	+	CPH	+	Chronic ALT elevation
13	19	7	2,112	Yes	+	CPH	+	Recovered, 4 years
14	>100	*	*	*	*	CPH	+	*
15	nr	nr	*	Yes	–	CPH	+	*
16	nr	nr	*	No	–	nd	nd	*
17	nr	nr	*	Yes	++	nd	+	*
18	nr	nr	*	Yes	–	CPH	+	*

Cases 1–14 had post-transfusion hepatitis and 15–18 were implicated donors; units, units of blood transfused; onset, weeks after transfusion; ALT = alanine tranferase enzyme; symptoms assessed as 0–4+; liver biopsy cited as chronic active hepatitis (CAH) or chronic persistent hepatitis (CPH); nd = not determined; nr = not relevant.

* Data unavailable.

granulocyte reactive ANA (G-ANA) (Hooper *et al.*, 1972), and cultured fibroblasts were used for antibodies to cytofilaments (Pedersen *et al.*, 1982). Antibody to LMAg was quantitated by a radiometric assay (Frazer, Kronborg & Mackay, 1983).

Histological interpretations. Percutaneous liver biopsies were available from most cases in the present study and the interpretations are by courtesy of Dr Kamal Ishak of the Armed Forces Institute of Pathology, Washington, D.C., USA. The biopsies were interpreted according to specific histological criteria, particularly for the designation of chronic active hepatitis or chronic persistent hepatitis (International Group, 1977). Although there is no definitive lesion specifying chronic NANB hepatitis, the biopsies were regarded as consistent with this diagnosis and not consistent with alcoholic, toxic or immunopathic causes.

Chimpanzee inoculation. Sera from nine of 18 patients included in this study had been proven to cause NANB infection in the chimpanzee. From 1 to 75 ml of serum or plasma from these patients were administered intravenously to chimpanzees who were followed weekly for at least 6 months for the development of transaminase (ALT) elevation, for light microscopic (LM) evidence of hepatitis and for electron microscopic (EM) evidence of ultrastructural changes thought characteristic of NANB (Shimizu *et al.*, 1979). The diagnosis of NANB was made when ALT elevation occurred between 2 and 26 weeks after inoculation without apparent non-viral cause and when this elevation was accompanied by LM and/or EM changes compatible with NANB hepatitis.

Classification of 18 NANB sera according to transmission data. Four of the sera were from donors whose blood was implicated in the transmission of NANB post-transfusion hepatitis: three had abnormal serum transaminase levels, liver biopsy available from two showed chronic persistent hepatitis and all four sera transmitted hepatitis to chimpanzees.

Twelve of the sera were from cases of chronic post-transfusion hepatitis who were prospectively followed with serum samples at 1–2 week intervals and in whom pre-transfusion transaminase levels were normal: liver biopsies available from 10 showed CAH in six, CAH undergoing resolution to CPH in three and CPH in one and all of four sera tested from this group transmitted infection to chimpanzees.

Two of the sera were from cases of acute post-transfusion hepatitis: a liver biopsy available later from one showed CPH and the one serum tested transmitted infection to a chimpanzee.

Table 2. Frequency of autoantibodies in chronic non-A, non-B hepatitis in post-transfusion subjects (PTH) in implicated blood donors and in controls*†

Category (No. of cases)	Positive tests for autoantibodies—titre ( )						
	ANA			ACFA			
	RL	G	ASMA	actin	IF	AMA	A-LMAg
PTH-NANB							
CAH (9)	1 (4)	0	0	0	0	0	0
CPH (5)	0	0	0	1 (16)	1 (16)	0	0
no biopsy (2)	0	0	0	0	1 (16)	0	nt
Implicated donors‡ (4)	0	0	0	0	1 (32)	0	nt
Total, NANB (18)	1	1	0	1	3	0	0/10
Internal controls (6)	0	0	0	0	0	1 (160)	0
Popn. controls (3,492)	2·4	6·4	1·5	nt	nt	<0·1	nt
A-CAH (52)	62	94	81	49	51	4	70

* For abbreviations, see text.

† Controls: the six 'internal' control sera were referred under code with the test panel; the normal population controls and the cases of autoimmune (A)-CAH were derived from earlier studies (referenced in text), and for these percentage frequency of positive results is cited.

‡ Biopsies performed in two showed CPH.

RESULTS

The results are shown in Table 2 for the categories of post-transfusion chronic hepatitis due to NANB (PTH-NANB), donors implicated in transmitting NANB hepatitis, and controls. The frequency of autoimmune serological reactions in these 18 cases of NANB chronic hepatitis was remarkably low. Two sera gave low titre (1/4) reactions for ANA, none gave reactions for ASMA and four gave low titre (1/4–1/16) reactions for antibody to cytofilaments, one anti-actin and three anti-intermediate filaments. Results for antibody to LMAg were all within the range for normal subjects. Serial tests on the four weak, autoantibody positive sera in this panel were not performed. For comparison, results are cited in Table 2 for three groups of 'Controls'. (i) 'Internal controls' refer to six sera included with the standard NANB panel but known not to contain an NANB agent; these included sera derived from normal subjects (four) and cases of primary biliary cirrhosis (one) and alcoholic cirrhosis (one). (ii) 'Population controls' cites data for normal Australian subjects (Hooper *et al.*, 1972) and are included to indicate results for the serological methods used in our laboratory—these data are representative of frequencies of autoantibodies for healthy populations of European origin (Mackay, Whittingham & Mathews, 1977). (iii) 'Autoimmune CAH controls' are included to provide data on the frequency of autoantibodies in this disease category by the serological methods used in our laboratory (Whittingham *et al.*, 1981; Pedersen *et al.*, 1982).

DISCUSSION

We have shown for a group of patients with chronic NANB viral hepatitis that the autoantibody markers characteristic of autoimmune CAH were conspicuously lacking. Although the number of cases of NANB in this study was not large, and some showed the liver lesions of chronic persistent rather than chronic active hepatitis, the documentation of the diagnosis of NANB hepatitis was as good as can currently be obtained. Thus all of the post-transfusion cases of NANB hepatitis were prospectively followed patients with normal serum transaminase levels pre-transfusion with the onset and persistence of abnormal serum enzyme levels fully documented, other forms of viral hepatitis were excluded, all sera so tested transmitted NANB hepatitis to chimpanzees, and liver biopsies helped to exclude alcoholic, toxic or immunopathic causes of chronic hepatitis. We considered it superior to test 'well pedigreed' cases of NANB hepatitis rather than a large number of cases of putative NANB hepatitis diagnosed only by the exclusion of markers of infection with hepatitis A or B virus.

The findings from the study showed a virtually clear serological segregation between autoimmune CAH, which is characterized by high titre reactions to autoantigens, particularly of nuclei and smooth muscle, and the present cases of chronic NANB hepatitis in which such reactions were either negative, or below the lowest of the range of titres, 1/20–1/80, cited by various authors as the 'cut off' for positivity for ANA and ASMA, thus being similar to data for our normal population. Also, antibody to liver membrane antigen, a further marker of autoimmune CAH, was negative in the 10 cases of NANB hepatitis tested, as found by others (Meyer zum Büschenfelde & Manns, 1984). There are very few studies on autoimmune serological reactions in groups of cases of NANB hepatitis, and in none was the diagnosis of NANB hepatitis confirmed by transmission studies. Negative reactions in acute NANB hepatitis were reported by Pedersen *et al.* (1981), Bretherton *et al.* (1983) and by Maier *et al.* (1983). Of 10 cases of biopsy proven cases of chronic NANB hepatitis occurring after blood transfusion, one positive result for ANA, one for AMA and none for SMA were reported, without titration data (Knodell, Conrad & Ishak, 1977). Tage-Jensen *et al.* (1980) described positive tests for autoantibodies in 10 cases of non-A, non-B chronic liver disease (10 females, median age 71 years) with six of 10 positive for SMA and nine of 10 positive for ANA; the possibility was conceded that 'this group comprised patients with a non-viral hepatitis with autoimmune manifestations'. Comment could be made on the reactivity of three of the present 18 NANB sera with cytoskeletal intermediate filaments, presumably vimentin. This reactivity is regarded as specifying viral rather than autoimmune hepatitis, since it has been reported in

association with various viral infections (Toh, 1979), including hepatitis A and B (Pedersen *et al.*, 1981; Bretherton *et al.*, 1983) and may be due to cross-reactivity between cytofilament and viral antigens (Fujinami *et al.*, 1983).

We conclude that among HBsAg negative cases of chronic hepatitis the autoimmune type of CAH is distinguishable from chronic liver disease due to infection with NANB viral agent(s) by a clear difference in frequency of serological reactivities with various tissue antigens. Practical conclusions from these results relate to prognosis and the decision to give long term treatment with prednisolone from which benefit can be expected in autoimmune CAH but probably not in NANB CAH.

We thank Dr Senga Whittingham and Miss J. Macgeorge for results of the serological assays. I. R. Mackay, I. H. Frazer, B.-H. Toh and J. S. Pedersen are in receipt of grants from the National Health and Medical Research Council of Australia.

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