NON-A, NON-B HEPATITIS OCCURRING IN AGAMMAGLOBULINAEMIC PATIENTS AFTER INTRAVENOUS IMMUNOGLOBULIN

A. M. L. LEVER A. D. B. WEBSTER
D. BROWN H. C. THOMAS

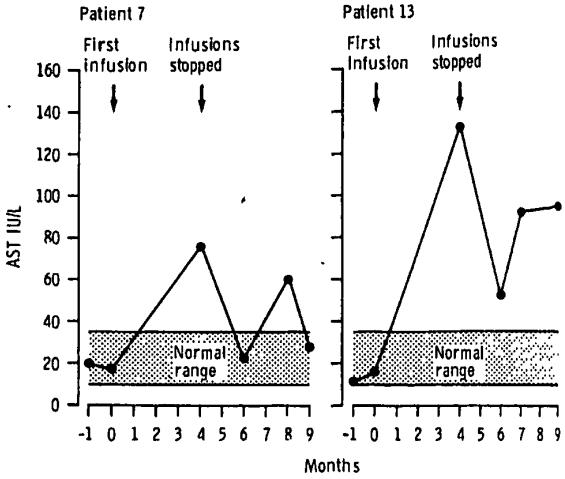
Division of Immunological Medicine, Clinical Research Centre, Harrow; and Department of Medicine, Royal Free Hospital, London

Summary Acute non-A, non-B hepatitis developed in twelve patients with primary hypogammaglobulinaemia during treatment with intravenous gammaglobulin prepared by Cohn fractionation of pooled plasma. The illness was clinically and histologically identical to the short-incubation non-A, non-B, hepatitis observed in haemophilic patients receiving factor VIII concentrates. Most of the patients were symptomless, but 10 months after onset ten of the twelve still had abnormal liver function. The occurrence of non-A, non-B hepatitis in agammaglobulinaemics indicates that humoral mechanisms are not essential for production of hepatocyte necrosis in this infection. This outbreak emphasises the need for a screening test to identify the agent in blood products, and shows that Cohn fractionation of plasma does not always inactivate the agent. Furthermore, the finding that the virus can be transmitted in IgG concentrates suggests either that the general population has a very low level of antibodies to the putative virus or that such antibodies are not virus-neutralising.

Introduction

SENSITIVE radioimmunoassays for hepatitis B surface antigen and IgM anti-HB-core allow identification of cases of post-transfusion hepatitis caused by the hepatitis B virus, and similar assays exist for the diagnosis of hepatitis A, cytomegalovirus, and Epstein-Barr virus infections which are rarer causes. Most post-transfusion hepatitis, however, is caused by a group of unidentified viruses designated non-A, non-B.[1] Serial investigations of haemophilic patients[2] and cross-challenge experiments in chimpanzees[3,4] have confirmed the existence of at least two parenterally transmitted non-A, non-B viruses with differing incubation periods. The short-incubation (2–4 weeks) type of non-A, non-B hepatitis is seen predominantly in haemophilic patients receiving factor VIII concentrates.[5] This disease is usually mild during the acute phase but a large proportion, usually greater than 80%, go on to acquire chronic lesions culminating sometimes in cirrhosis.[5] The serum transaminases fluctuate rapidly during the course of this condition and liver biopsy usually reveals a lobular hepatitis in which the mononuclear cell infiltrate is disproportionately high in relation to hepatocyte necrosis.[5] The second type of parenterally transmitted non-A, non-B hepatitis has an incubation period of 6–10 weeks. This also is a mild illness but 20–40% of patients still have abnormal liver function 6 months after onset,[6] progressing sometimes to cirrhosis. Again, the transaminases may fluctuate and liver biopsy shows chronic persistent or chronic active hepatitis with only minor lobular inflammation. This is a point of distinction from the short-incubation haemophilia-associated type of disease.

In contrast to plasma, blood, and coagulation factors, immunoglobulin preparations have rarely transmitted hepatitis. Because immunoglobulin preparations are usually prepared from large plasma pools derived from numerous donors, it has been assumed that the Cohn fractionation process either excludes or inactivates viruses.[7] We describe here an outbreak of non-A, non-B hepatitis acquired from Cohn fraction II material modified for intravenous injection. The study helps clarify the role of specific antibody in the pathogenesis of this condition.



Aspartate aminotransferase fluctuations in two representative patients.

Patients and Methods

Twenty-four patients with hypogammaglobulinaemia were recruited into an open cross-over trial to evaluate the efficacy of intravenous gammaglobulin versus conventional intramuscular immunoglobulin in preventing infection. Twelve patients were allocated to intravenous treatment, three of them with X-linked agammaglobulnaemia and 9 (2 females) with "common variable" hypogammaglobulinaemia. All twelve had previously been on regular weekly intramuscular gammaglobulin replacement therapy (25-50 mg/kg). Three had also been receiving two units of plasma every three weeks. All patients had normal serum transaminase levels at the start of the trial.

The intravenous globulin was prepared by the British Blood Products Laboratory by conventional[8] alcohol fractionation. Maltose was added to stabilise the immunoglobulin, followed by removal of the alcohol on a Sephadex G25 column and 0·2 μm filtration; the resulting solution was freeze dried. It was given fortnightly, freshly reconstituted with pyrogen-free water, at a dose of 200 mg/kg and patients were monitored for adverse effects. The trial was discontinued when hepatitis developed in some of the patients.

Peripheral-blood T cell numbers and T cell markers for helper and suppressor/cytotoxic cells were measured with commercial antibodies (Leu 1, 2, 3a) on a fluorescence activated cell sorter. Cytotoxic (NK) cell activity was assessed with a chromium-51 release assay with myeloid cells (K562) as targets, and lymphocyte/target cell ratios ranging from 1:1 to 200:1.[9] Concanavalin-A-induced suppressor function was measured in vitro with Con A concentrations of 10 μg, 5 μg, and 1 μg per well.[10] All tests were done on freshly separated peripheral-blood mononuclear cells or on samples that had been frozen in liquid nitrogen within half an hour of separation (this freezing technique has been shown not to influence either lymphocyte populations or functional assays).

Liver function tests were done before the trial and then monthly after the onset of hepatitis for a total of 10 months. In the three patients who had a liver biopsy (Menghini needle) the samples were sent for conventional histological examination and serial sections were examined for hepatitis B surface antigen. Hepatitis B surface antigen was measured in the serum of all patients with a commercial assay (Abbott).

Results

Clinical Observations

Within two weeks of the first infusion, one patient who had previously been receiving plasma experienced a "flu-like" illness and two weeks later became jaundiced with greatly raised transaminase concentrations. Hepatitis B surface antigen was absent from the blood and no virus particles were detected on electronmicroscopy of the stools. Non-A, non-B hepatitis was provisionally diagnosed. The other recipients of the intravenous immunoglobulin preparations were clinically well at this time and showed symptomatic benefit from their higher serum immunoglobulin concentrations. Therefore it was assumed that the patient with non-A, non-B hepatitis had acquired the virus from previous plasma therapy. However, 3 months after the onset of the trial, the patients were reassessed and all proved to have a raised serum aspartate aminotransferase. One patient, on close questioning, admitted to having been mildly jaundiced for about a week between infusions. Three patients then had a liver biopsy.

Six months after diagnosis of hepatitis all patients had raised transaminase concentrations and were thus, by definition, at the stage of chronic hepatitis. The transaminases have since returned to normal in two. Only two of the group ever became clinically jaundiced and most were symptomless. One patient now has unexplained marrow hypoplasia.

Laboratory Tests

The baseline aspartate aminotransferase concentrations were all normal (10–35 IU/ml), and at the first assessment after treatment all were abnormal (mean 132, range 39–545). Some patients had large fluctuations in transaminases (figure).

Hepatitis B surface antigen was never detected in any patient. Table I shows T-cell helper/suppressor ratios in the twelve patients who received intravenous gammaglobulin and in four patients who were on intramuscular gammaglobulin originating from the same plasma pool as the intravenous preparation. Where the T cell phenotype had been examined previously, this is recorded. Overall there is little or no change in subset ratios and no reversal of the ratios. Those patients who were known to have excess suppressor cells before the trial started continued to show such an excess. Concanavalin-A-induced T suppressor activity, measured in patients 2, 4, and 8, was normal (table II). NK cell activity, measured in the same three subjects, was low in patient 4 but normal in patients 2 and 8 (table III).

All three biopsy specimens showed severe lobular hepatitis with widespread mononuclear cell infiltration of the hepatic sinusoids. There was reticulin condensation indicating some liver cell loss, but in general the inflammatory infiltrate was disproportionate to the amount of liver cell necrosis.

TABLE I—T CELL PHENOTYPE

Patient	Pan-T (Leu-1) %	Helper T (Leu-3) %	Suppressor T (Leu-2) %	Ratio*	1981 ratio*
Intravenous gamma-globulin					
1	78	29	58	0·5	0·37
2	83	59	36	1·64	ND
3	29	25	19	1·3	ND
4	82	50	44	1·14	0·72
5	71	44	39	1·13	1·2
6	26	19	5	3·8	4·8
7	57	43	19	2·26	2·8
8	29	9	20	0·45	ND
Intramuscular gamma-globulin					
9	41	33	15	2·2	1·24
10	58	44	26	1·7	1·77
11	52	41	31	1·3	1·83
12	70	28	29	0·96	0·76

*Normal = 1·1–3·1.
ND = not done.

TABLE II—CON A SUPPRESSOR ACTIVITY

—	Patients 2	4	8	NR±SD
Con A concentration/well				
10 μg	0·81	1·08	1·16	1·1±0·13
5 μg	1·15	2·55	1·53	1·3±0·41
1 μg	0·63	4·39	1·64	2·9±0·7

TABLE III—NK CELL ACTIVITY (% CYTOTOXICITY)

—	Patients 2	4	8	NR±SD
Lymphocyte: target cell ratio				
200	66·3	23	61·5	69·4±13·5
100	57·5	17·5	49	74·14±7·61
50	21·1	12·3	38·9	62·5±17·6
10	12·7	4·6	15·3	28·6±9·1
1	2·5	1·0	2·9	5·1±2·4

NR = normal range.

Discussion

All the hypogammaglobulinaemic patients who received intravenous gammaglobulin acquired a short-incubation non-A, non-B hepatitis which progressed to chronic hepatitis. The rapidity of onset (2–4 weeks after infusion), the high chronicity rate, and the prominent lobular hepatitis seen on liver biopsy were reminiscent of the short-incubation non-A, non-B hepatitis seen in haemophilic patients receiving factor VIII concentrate. Probably the same virus is responsible for both conditions.

The fact that large-pool gammaglobulin preparations are capable of transmitting this type of non-A, non-B hepatitis implies that, in the community, virus-neutralising antibody occurs rarely or in extremely low titre. This has already been suggested by the observation that pooled intravenous immunoglobulin preparations do not prevent non-A, non-B hepatitis infection transmitted by factor VIII concentrates to haemophilic patients[11] and experimentally to chimpanzees.[12] Furthermore, the occurrence of severe non-A, non-B hepatitis in agammaglobulinaemic patients suggests that humoral immune mechanisms are not involved in the liver cell damage; we presume that the mechanisms are cellular or that the virus is directly cytopathic. In our patients T cell and NK function were normal and the prominent cellular infiltrate in the liver suggests their participation in the process. This notion is supported by the observation that the mononuclear cells in the livers of haemophilic patients are predominantly of the T8 phenotype (H. C. Thomas,

unpublished) which include cytotoxic T cells. There were also no significant changes in T-cell subset ratios—ie, the excess of circulating suppressor T8 lymphocytes in some hypogammaglobulinaemic patients is unlikely to be due to subclinical non-A, non-B virus infection acquired from gammaglobulin treatment.

Bone-marrow aplasia developed in one patient. This is of particular interest in that anaemia has been reported in a chimpanzee with short-incubation non-A, non-B hepatitis after injections of factor VIII concentrate.[13] Human parvovirus, which causes a failure of erythropoiesis in sickle cell anaemia,[14] can be transmitted by factor VIII concentrates[15] and can cause hepatitis. Diagnosis of parvovirus infections depends on detection of a specific IgM response which in hypogammaglobulinaemic patients is not possible. Parvovirus antigen was sought in the patient with marrow aplasia and not detected, but a parvovirus-like agent does remain a possible cause of this particular outbreak.

The apparent absence of hepatitis in patients receiving intramuscular immunoglobulin from the same pool of plasma suggests that minor variations in the manufacturing process allowed the non-A, non-B virus to remain infectious in the intravenous preparation.[16] The dose and route of administration of the infected material, and the susceptibility of the host, may also have influenced the infection rate and severity of the disease. Other intravenous gammaglobulin preparations currently available do not seem to have transmitted hepatitis, probably because viruses are inactivated by the procedures used. Various other methods have been suggested to eliminate hepatitis virus in blood products.[17,18]

Correspondence should be addressed to A. M. L. L., Division of Immunological Medicine, Clinical Research Centre, Harrow, Middlesex HA1 3UJ.

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INFLUENCE OF PROPRANOLOL ON WEIGHT AND SALT AND WATER HOMOEOSTASIS IN CHRONIC LIVER DISEASE

P. C. HAYES W. W. STEWART
I. A. D. BOUCHIER

University Department of Medicine and Department of Medical Physics, Ninewells Hospital and Medical School, Dundee

Summary Sixteen patients with chronic liver disease were treated with propranolol or placebo in a double-blind study. Skinfold thickness, total body water and exchangeable sodium, and urinary sodium were measured every 6 months for a year; body weight was measured every 2 (later every 3) months for 15 months. Propranolol treatment was associated with a significant rise in body weight after 9 months and significant rises in skinfold thickness and body fat after 12 months. Propranolol-treated patients showed a fall in total body water at 6 months and a rise in urinary sodium concentration at 12 months. They did not show the rise in total body exchangeable sodium that occurred in placebo-treated patients. Propranolol seems to affect salt and water homoeostasis favourably in patients with chronic liver disease.

Introduction

SODIUM retention is a characteristic of advanced liver disease, but the mechanism is unclear. Activation of the renin-angiotensin-aldosterone system, which occurs in patients with ascites,[1–4] may be important. Plasma renin levels correlate directly with the wedged hepatic venous pressure.[5] Increased sympathetic activity has been implicated in the impaired sodium and water excretion in cirrhotic patients.[6] Animal models suggest that sodium retention precedes ascites formation,[7,8] although this view is not universally supported.[9] Associated with the increase in body sodium and water is a loss of body fat and cellular mass.[10]

The effect of propranolol, advocated by Lebrec et al[11] for prevention of rebleeding from oesophageal varices in cirrhotic patients, on the renin-angiotensin system in patients with cirrhosis and ascites has been studied. Wilkinson et al showed that propranolol protected against diuretic-induced renal impairment in cirrhotic patients by blocking the rise in plasma renin activity.[12] Despite suppression of the plasma renin activity in cirrhotic patients with ascites treated with β-adrenergic antagonists, the renal excretion of aldosterone is variable, suggesting the involvement of other factors.[2] Renal sodium excretion is inversely related to aldosterone excretion before and after β-adrenergic blockade.[2]

In a study of four patients with cirrhosis and ascites, Panitch and co-workers observed a rise in urinary sodium in a patient whose renin and aldosterone levels returned to normal on propranolol.[3] Shohat et al found that propranolol increased urinary sodium excretion after an acute saline load in five cirrhotic patients with ascites.[13] This finding is

A. M. L. LEVER AND OTHERS: REFERENCES—*continued*

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