Hepatitis G virus infection as a possible causative agent of community-acquired hepatitis and associated aplastic anaemia

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Summary

Aplastic anaemia complicating hepatitis is a rare but well-documented phenomenon; however in many patients the cause remains unknown. We present a 24-year-old man with a well-defined community-acquired hepatitis, probably due to hepatitis G virus (HGV), who developed severe aplastic anaemia. In this case, the absence of other agents likely to cause the clinical manifestations, and the detection of HGV RNA at the time of illness, clearly point to this agent as being responsible for both the hepatitis and the aplastic anaemia. Further studies in serial serum samples and meticulous evaluation of the disorders associated with the infection will be needed to prove or dispute a causal association of HGV and aplastic anaemia.

Keywords: hepatitis G virus; aplastic anaemia

Aplastic anaemia complicating hepatitis is a rare but well-documented phenomenon. The mechanisms leading to marrow failure remain unclear.^{1,2} The recent identification of the hepatitis G virus (HGV) has opened the field to the study of disease association with these new agents.^{3,4} HGV-RNA has been reported in sera from patients with acute, chronic, and fulminant hepatitis who lacked evidence of infection with known hepatitis viruses.^{3,4} Of interest is an apparent link between HGV infection and cases of aplastic anaemia.^{5,6}

Case report

On 10 July 1992, a previously healthy white 24-year-old heterosexual man presented with 5 weeks of malaise and 3 weeks of jaundice. The patient had had no exposure to industrial chemicals or to any proprietary or non-proprietary drugs. He denied use of any medications, intravenous drug abuse, blood transfusion, or alcohol ingestion. On examination, the patient was alert with minimal scleral icterus and without stigmata of chronic liver disease. Serologic markers for HAV, HBV, HCV, HEV, and HIV were repeatedly negative. HBV and HCV testing by polymerase chain reaction (PCR) did not detect HBV-DNA or HCV-RNA. Virological and microbiological tests, including cytomegalovirus, Parvovirus B-19 (IgG and IgM), toxoplasma, Epstein-Barr virus, herpes simplex virus, coxiella, and leptospira, were all negative. Antinuclear antibodies, anti-smooth muscle antibody, antimitochondrial antibody, and screen for Wilson's disease and haemochromatosis were also negative. Chest X-ray and ultrasound examination of the abdomen were normal. His clinical state improved over the next 10 weeks; liver function tests returned to normal levels by week 12. On 5 August 1993 he was admitted to our hospital for evaluation of nose and gum bleeding and multiple haematomata. Examination revealed purpura of his oral mucosa and multiple petechiae. Virological and microbiological tests were again negative. Bone marrow biopsy showed a severely hypoplastic marrow and this, together with his low platelet and white cell counts, satisfied the criteria for severe aplastic anaemia. Over the following 2 weeks, he became progressively more pancytopenic and his aspartate transaminase and alanine transaminase returned to near normal ranges. On 25 August 1993 he received an allogeneic bone marrow transplant. He has remained well for more than 2 years after transplantation. The laboratory results are summarised in table 1.

HCV-RNA was investigated by PCR amplification of the 5' NCR as described previously.⁷ No cross-reactivity with HGV-RNA was found. HGV-RNA was investigated by PCR amplification of the 5' NCR as described previously.⁸ Briefly, nucleic acids were extracted from 100 µl of serum using extraction columns (Quiagen, Hilden, Germany). RNA was transcribed into cDNA with M-MLV retrotranscriptase. The primers were NCRI:

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Table 1 The haematological, biochemical, serological, and virological course of the community-acquired hepatitis and associated aplastic anaemia

Variable	July 1992	Sep 1992	Aug 1993	July 1994	Dec 1994
Hemoglobin (g/l)	151	145	100	135	137
White cells ($\times 10^9/l$)	6.1	7.35	1.3	5.45	6.7
Platelets ($\times 10^9/l$)	310	254	10	221	236
^a PI (%)	100	100	80	100	100
^b AST (IU/l)	941	40	451	51	35
^c ALT (IU/l)	1812	45	1344	70	38
^d AP (IU/l)	190	83	185	95	75
^e GGT (IU/l)	364	45	218	40	45
^f TB (μ mol/l)	51.3	22	55.3	27.2	31.2
^g HGV-RNA	Positive	Positive	Positive	Negative	Negative

^aPI: Prothrombin index (normal value 70–100%); ^bAST: aspartate transaminase (normal value 10–40 IU/l); ^cALT: alanine transaminase (10–40 IU/l); ^dAP: alkaline phosphatase (40–120 IU/l); ^eGGT: gamma-glutamyltranspeptidase (10–50 IU/l); ^fTB: total bilirubin (3.42–17.1 μ mol/l); ^gHGV-RNA detected by polymerase chain reaction.

5'-CGGCCAAAAGGTGGTGGATG-3' and NCR2: 5'-CGACGAGCCTGACGTC GGG-3'. Amplified cDNA sequences were detected by agarose gel electrophoresis with ethidium bromide staining. The specificity of the amplified bands was confirmed by an electro-immunoassay procedure (PCR ELISA (Dig detection, Boehringer Mannheim, Germany). Sera obtained before any blood products were given and throughout the clinical course, were positive for HGV. Similarly, serum obtained on presentation with aplastic anaemia was positive for HGV. Unfortunately, fresh bone marrow aspirates were not available to show whether HGV RNA was present in bone marrow. Sera obtained in July and December 1994, were negative for HGV.

Discussion

Hepatitis-associated aplastic anaemia (HAAA) is a severe disorder with a high mortality; the responsible agent for most cases of HAAA has not been identified, although it is presumed to be viral. HGV has been postulated as the aetiological agent of acute hepatitis, chronic liver disease of unknown aetiology, fulminant hepatitis, and HAAA.^{3,4} In our patient, we were able to detect HGV-RNA in the serum repeatedly before any blood transfusion. In this case, the HGV infection was demonstrated coincident with the two flares of hepatic necrosis, and for this reason a causative association can be inferred. The role of HGV in non-A-E community-acquired hepatitis is under investigation.⁹⁻¹¹ Published case series have claimed an association between HGV and acute liver diseases, but case-control studies are required to confirm whether or not HGV is an aetiological agent of viral hepatitis.⁹ The negative HGV RNA by PCR in the post-bone marrow transplant serum suggests HGV clearance in this patient. Few reports have analysed the role of HGV in the development of HAAA (table 2); however, the high prevalence of HGV in these cases may arise from disease treatment rather than from HGV being a causative agent of aplastic anaemia.

Table 2 Cases reported of possible HGV infection as a causative agent of hepatitis-associated aplastic anaemia

Reference	n patients	HGV-RNA positive (%)
1	10	3 (30)
3	1	1 (100)
5	1	1 (100)
6	1	1 (100)
10	4	4 (100)
present case	1	1 (100)

The patient with HGV hepatitis we report here followed a pattern noted to be typical of HAAA. Moreover, after the bone marrow transplant, there was no further evidence of chronic hepatitis or HGV infection. Although the detection of HGV RNA at the time of illness, in the absence of other agents likely to cause the clinical manifestations, clearly points to this agent as being responsible for both the hepatitis and the aplastic anaemia, controversial issues should be addressed. First, the clinical significance of HGV remains uncertain.⁸⁻¹⁰ Second, neither a specific location nor replication in the liver has yet been verified and HGV RNA has not been documented in fresh marrow aspirates. Third, we cannot exclude that our patient with HGV infection may have simultaneously acquired another unidentified agent(s) which might have caused the rise in liver enzymes and/or the aplastic anaemia. Fourth, several features of the HAAA suggest that it is mediated by immunopathologic mechanisms; for this reason, the causative agent of this syndrome may not be an infectious agent.¹⁻²

In summary, in this case the absence of other agents likely to cause the clinical manifestations, and the detection of HGV RNA at the time of illness, clearly point to this agent as being responsible for both the hepatitis and the aplastic anaemia.

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Primary antibody deficiency and Crohn's disease

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Summary

Five patients with primary antibody deficiency were investigated because of intermittent but persistent diarrhoea of several years duration despite immunoglobulin replacement therapy. We found no evidence of *Giardia lamblia* or other intestinal pathogens to explain their gastrointestinal symptoms. All five had definite radiological evidence of small bowel Crohn's disease and three had histological specimens available with abnormalities consistent with Crohn's disease. One patient had a non-caseating granuloma in an oral ulcer. A second patient with stricturing disease in the small bowel had a mucosal inflammatory infiltrate with non-caseating granulomas. A third had transmural inflammation but no granulomas. All five patients were diagnosed as having Crohn's disease and have responded symptomatically to steroid therapy.

Keywords: antibody deficiency; Crohn's disease

Some patients with primary antibody deficiency have an enteropathy. Infection is common and although immunoglobulin replacement therapy seems to have reduced the incidence of some bowel pathogens such as *Giardia*, *Salmonella* and *Campylobacter*, 10% have diarrhoea, abdominal pain or weight loss which defies explanation.¹

There is a broad spectrum of histological abnormality in these patients. In the stomach there is a high frequency of gastric atrophy and a predisposition to gastric carcinoma,²⁻⁴ whilst in the small bowel, lymphoid nodular hyperplasia is common.²⁻⁵ In those with common variable immunodeficiency, there are well-documented cases of jejunal villous atrophy which may or may not respond to gluten withdrawal; however, the diagnosis of coeliac disease has only been reliably proved in one patient after gluten withdrawal and challenge.⁶

There have been several case reports showing radiological appearances compatible with Crohn's disease in patients who have presented with diarrhoea and intestinal strictures, although the histology has generally not shown granulomas or transmural inflammation.⁷⁻¹¹ Teahon and Webster,¹¹ in their series of patients with primary antibody deficiency, proposed the existence of an enteropathy distinct from Crohn's disease. The radiology was consistent

with Crohn's disease but the histology was apparently distinctive with the presence of microscopic colitis, increased intra-epithelial lymphocytes and an intact crypt architecture in the colon. Any inflammation in the small bowel was not transmural and there were no granulomas.

We investigated the cause of diarrhoea in five patients with primary antibody deficiency who were referred with refractory diarrhoea.

Methods

We investigated five patients who were referred with intermittent but persistent diarrhoea of several years duration from a cohort of 100 patients with primary antibody deficiency from the Department of Immunology at Hope Hospital in Manchester over a period of 8 years. There were four men (19-65 years) and one woman (54 years). One had X-linked agammaglobulinaemia (Bruton's disease), the others had common variable immunodeficiency. All five patients had been shown to have a primary immunological deficiency by their repeated failure to generate responses to common antigens such as pneumococcus, tetanus and pokeweed nitrogen. All were on regular immunoglobulin replacement therapy with Sandoglobulin (Sandoz Pharmaceuticals) at a dose of 0.3-0.4 g/kg/month with regular assessment of their immunoglobulin trough levels.

Two sets of three fresh stool specimens from each patient were examined for evidence of parasitic involvement or other pathogens. Each patient had a gastroscopy with duodenal biopsy, full colonoscopy and a small bowel enema. The specimens obtained were examined histologically for parasites.

Patients were commenced on steroid therapy and their response assessed according to improvements in their symptoms. They were then followed up over a 5-year period.

Results

The main findings are shown the table. No pathogens were identified from several stool examinations specifically looking for *Salmonella*, *Shigella*, *Campylobacter*, ova and cysts. All of the patients had radiological evidence of small bowel abnormalities compatible with Crohn's disease. Patient 1 had nodular thickening throughout the jejunum and ileum (figures 1 and 2). Patient 2 showed stricturing disease in the duodenum and small bowel (figure 3). Patient 3 had a stenotic lesion in the terminal

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