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Case report

Immune-mediated hepatitis-associated aplastic anemia caused by the emergence of a mutant hepatitis B virus undetectable by standard assays☆

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Hepatitis-associated aplastic anemia (HAA) is characterized by marrow failure developing after acute seronegative hepatitis. A patient with agammaglobulinemia developed HAA in association with HBsAg-negative, hepatitis B virus (HBV) DNA-positive acute hepatitis. Sequence analysis showed several substitutions in the major antigenic determinant of HBsAg, potentially affecting the detection by diagnostic immunoassays. Viral mutants may therefore be implicated as etiologic agents of HBsAg-negative HAA. HBV DNA determination may be necessary to exclude mutant HBV as a cause of HAA, particularly in categories at high risk of mutant selection such as agammaglobulinemic and transplanted patients.

***Keywords*: Aplastic anemia; Agammaglobulinemia; Hepatitis B virus (HBV); HBV mutant; HBsAg; Immunoassay; Antilymphocyte globulin**

1. Introduction

Hepatitis-associated aplastic anemia (HAA) is a well-recognized clinical syndrome in which marrow failure develops after acute hepatitis [1]. While several features of the syndrome suggest that its pathogenesis may be mediated by immunologic mechanisms, the etiologic agent of hepatitis has not been recognized yet and none of the known hepatitis viruses has been convincingly associated with HAA [2]. However, although the diagnostic accuracy of serological assays is continuously improving, false negative results cannot be ruled out.

The hepatitis B surface antigen (HBsAg) is the primary serological marker for the diagnosis of HBV infection. HBsAg is a 226-amino acid (aa) protein target of neutralizing antibody response mainly directed against the highly conformational hydrophilic domain located from aa 100 to 160, referred to as the *a* determinant. The selection of mutants harbouring aa substitutions within this region may reduce and even abolish the binding of HBsAg to the antibodies used in diagnostic assays. Viral variants with aa substitutions in the *a* determinant have been shown to emerge in vaccinated infants born to HBsAg-positive mothers, with or without concurrent administration of hepatitis B immune globulin (HBIg) [3], after liver transplantation and passive immunization with HBIg [4], or during the natural

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course of chronic infection [5,6] under the selective pressure of the patient's immune response.

Herein we document a case of HAA that developed in strict temporal association with a flare of HBV infection by a HBsAg-mutant virus, which could not be detected by HBsAg assays. Pancytopenia did not resolve when viral replication was controlled by lamivudine treatment but promptly subsided after immunosuppressive treatment with antilymphocyte globulin and cyclosporine A.

2. Materials and methods

2.1. Serological and immunological analysis

HBsAg was determined routinely by a screening (test A; AxSYM, Abbott Laboratories, North Chicago, IL) and a confirmatory (test B; VITROS ECi HBsAg, Ortho Clinical Diagnostics, Raritan, NJ) immunoassay. The specificity of HBsAg detection was tested by a neutralization assay (AxSYM). HBeAg, anti-HBc, anti-HBe, anti-HBs, anti-HCV, anti-HAV (IgM and IgG) were determined by AxSYM. Anti-HDV IgM and IgG were tested by ETI-DELTAK-IGMK-2 and ETI-AB-DELTAK-2 (DiaSorin, Saluggia, Italy), respectively. HBV DNA load was determined by the Versant HBV 3.0 assay (Bayer HealthCare, Tarrytown, NY; sensitivity limit: 2000 copies/ml). HCV RNA determination was carried out by the COBAS Amplicor (Roche Diagnostics, Basel, Switzerland) with a detection threshold of 50 IU/ml.

Peripheral blood lymphocytes were tested for T-cell receptor (TCR) diversity by heteroduplex analysis [7] and for thymus function by TCR excision circles (TRECs) production by real-time polymerase chain reaction (PCR) [8].

2.2. Nucleotide sequencing

DNA was extracted from serum with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). HBV S gene was amplified by PCR for 40 cycles (94 °C 30″, 58 °C 30″, 72 °C 1′), with primers B1F (5′-GTTGCCCGTTTGTCCTCTAATT-3′, nucleotide positions (nt) 465–486), and R4 (5′-GCCAAACAGTGGGGGAAAG-3′, nt 730–712), and directly sequenced by the Big Dye Terminator sequencing kit on an ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA). Hemi-nested PCR (sensitivity limit: 250 HBV DNA copies/ml) was performed with first step primers B1F-R7 (5′-CCCCAATACCAC ATCATCCATATA-3′, nt 761–738), and second step primers B1F-R4, as described above, for 35 and 25 cycles, respectively.

2.3. Case description

A 47-year-old male affected by chronic hypogammaglobulinemia persisting after resection of a thymoma had been receiving regular intravenous immunoglobulin (IVIg) prophylaxis for 4 years. Since January 2005 he progressively developed severe, transfusion-requiring anemia. The last IVIg infusion was administered on March 9th, 2005. On March 31st a marrow trephine biopsy showed pure red cell aplasia (PRCA) in a normocellular marrow with normal myeloid and megakaryocyte series. Pre-transfusion serum sample was negative for anti-HCV, HBsAg, HBeAg, anti-HBe, HBV DNA (hemi-nested PCR), and positive for anti-HBc and anti-HBs (173 mU/ml). Prednisone 1 mg/kg obtained partial resolution of anemia and transient transfusion independence. A repeat bone marrow biopsy showed the reappearance of the erythroid series (myeloid/erythroid ratio: 1/1) and moderate trilineage marrow hypoplasia with 20% cellularity. Prednisone was discontinued. After 1 month, a transaminase flare was detected and HBsAg testing led to discrepant results: the screening assay (test A, Fig. 1) was positive, whereas both the confirmatory (test B) and the neutralization assays scored negative. The result of test A

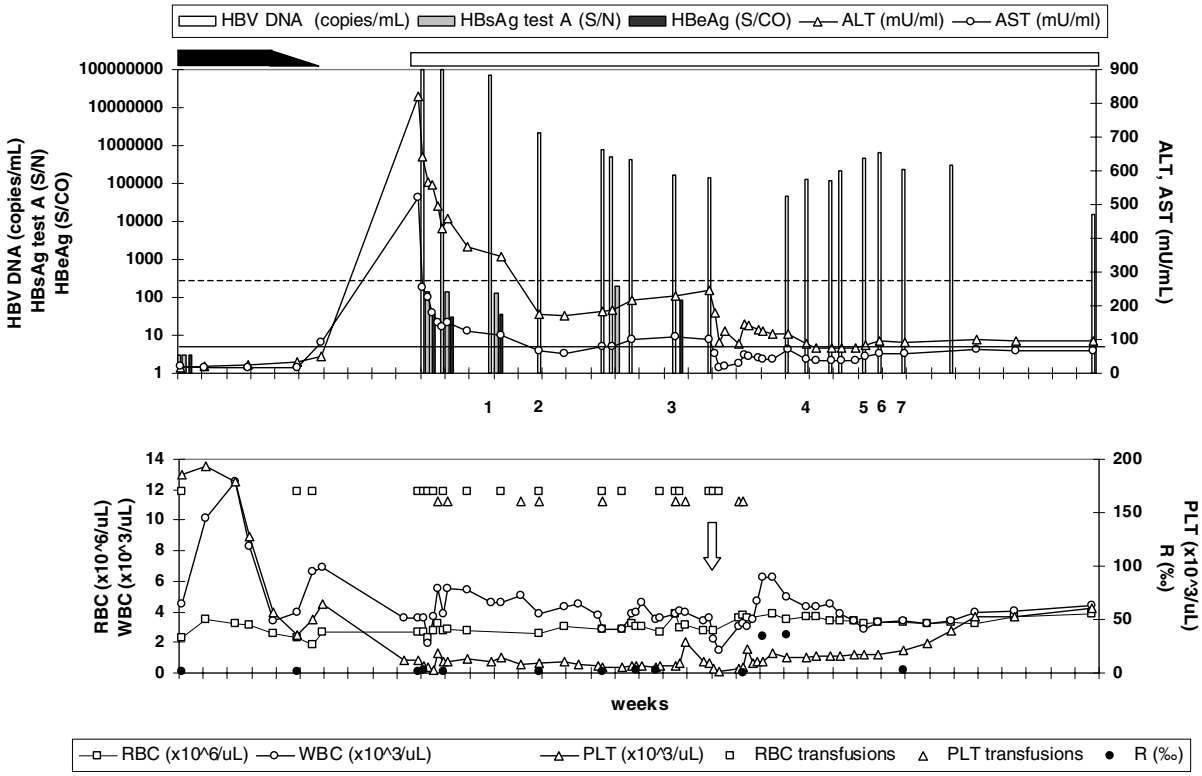

Fig. 1. Time course of biochemical and serological markers. ALT, alanine aminotransferase; AST, aspartate aminotransferase; RBC, red blood cells; WBC, white blood cells; R, reticulocytes; PLT, platelets. Black bar: steroid treatment. Blank bar: lamivudine treatment. The blank arrow indicates ALG administration. Dashed line: cut-off of HBV DNA assay. Continuos line: cut-off of serological assays. Serum samples analyzed by sequencing are numbered (1–7).