Significant Liver Injury with Dual Positive IgM Antibody to Epstein-Barr Virus and Cytomegalovirus as a Puzzling Initial Manifestation of Infectious Mononucleosis

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Abstract

A 35-year-old man was admitted because of significant hepatic dysfunction with mild splenomegaly and intra-abdominal lymphadenopathy of unknown cause. Infectious mononucleosis was suggested by subsequently detected high fever, pharyngotonsillitis and cervical lymphadenopathy, but IgM to Epstein-Barr virus (EBV) and cytomegalovirus (CMV) showed dual positivity. A definite diagnosis of EBV-induced infectious mononucleosis was established 3 months later on the basis of seroconversion to Epstein-Barr nuclear antigen (EBNA)-IgG positivity and reduced CMV-IgM titer with persistently negative CMV-IgG. This case highlights the initial diagnostic difficulties of EBV-induced infectious mononucleosis particularly in older patients, due to concomitant abnormal humoral immunity and unusual initial manifestations such as significant liver injury and extensive intra-abdominal lymphadenopathy.
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Key words: false positive IgM to cytomegalovirus, cross reaction, abdominal lymphadenopathy, multi-positive viral serologies, older patient of infectious mononucleosis, liver injury associated with infectious mononucleosis

Introduction

Infectious mononucleosis (IM) due to primary Epstein-Barr virus (EBV) infection commonly affects young adults between 15 and 30 years of age (1). Difficulties of initial diagnosis of IM in older individuals have been documented because of the rare occurrence, absence of typical IM manifestations such as lymphadenopathy, tonsillopharyngitis, lymphocytosis accompanied by atypical lymphocytes and heterophile-antibody (1). On the other hand, other pathogenic microorganisms such as *Toxoplasma* or viruses such as cytomegalovirus (CMV), human herpesvirus 6 (HHV-6) and human immunodeficiency virus (HIV), also provoke heterophile antibody-negative IM-like symptoms (1, 2). Further, it is also reported that EBV infection alters humoral immunity, i.e. infected B lymphocytes transform to proliferate indefinitely and secrete antibodies either to viral proteins or to host-cells (3), and occasionally present multi-positive serological results for viruses including CMV, HHV-6, measles virus and rubella virus (4, 5). These conditions make the prompt and correct diagnosis of EBV-induced IM problematic. We present here a patient who was atypical for IM with respect to age and the initial manifestations such as significant hepatic dysfunction, extensive intra-abdominal lymphadenopathy and dual positive immunoglobulin (Ig)M both to EBV and CMV. Diagnostic problems posed by older IM patients, the possible mechanism of double positive viral serological and the significance of enlarged intra-abdominal lymph nodes are also discussed.

Case Report

A 35-year-old previously healthy Japanese man presented in May 2002 because of malaise lasting for 7 days. The patient was afebrile at 36.8°C and the physical examination produced no unusual findings, not even superficial lymphadenopathy. Laboratory tests detected significantly elevated liver enzymes with aspartate aminotransferase (AST) 246 IU/*l* (5–35), alanine aminotransferase (ALT) 384 IU/*l* (5–30), alkaline phosphatase 470 IU/*l* (74–230), gamma-glutamyl transpeptidase 430 IU/*l* (5–45) and lactate

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dehydrogenase 1,258 IU/*l* (190–430). Total bilirubin was less than 0.6 mg/dl throughout the clinical course. Surface antigen of hepatitis B virus (HBV), IgM antibody to hepatitis A virus or to HBV core protein and antibody of hepatitis C virus were all negative. Repeated blood examinations also proved HBV surface antigen and HCV antibody negative. Inflammatory reaction or leukocytosis was absent, with a white blood cell (WBC) count of 6,100/μl (36% neutrophils, 55% lymphocytes, 7% atypical lymphocytes) and 0.4 mg/dl of C-reactive protein. Ultrasonography (US) and enhanced computed tomography (CT) showed splenomegaly and extensive intra-abdominal lymphadenopathy of the portocaval, peripancreatic and para-aortic lymph nodes (Fig. 1). The patient was suspected of acute hepatitis or lymphoproliferative disease on admission. Liver dysfunction reached a peak on the fourth hospital day as indicated by AST 389 IU/*l* and ALT 685 IU/*l*. On the sixth hospital day, the patient first developed a fever to 38.6°C and a sore throat, while severe exudative tonsillopharyngitis, and cervical lymphadenopathy developed rapidly, which prompted us to suspect IM. The WBC count peaked at that time, but the differential count was normal (10,400/μl of WBC count with 31% neutrophils, 55% lymphocytes, 12% monocytes, 1% basophils and 1% atypical lymphocytes). On the eighth hospital day, IgM antibodies with dual positivity to the viral capsid antigen of EBV and CMV were detected in the serum obtained at admission. This made IM highly likely, but led to diagnostic confusion as to which virus was responsible for IM symptoms. IM symptoms and intra-abdominal lymphadenopathy subsided as the result of only supportive therapies. When the patient left our hospital on the sixteenth day, IgM-CMV had become indeterminate in contrast to the unchanged titer of IgM-EBV (Table 1). About 3 months later, the patient was finally diagnosed as EBV-induced IM on the basis of seroconversion of Epstein-Barr nuclear antigen (EBNA)-IgG and persistent negative IgG-CMV. In this case, inactivated CMV, cultured with human cells, was used as the antigen for the detection of CMV-IgM by enzyme immunoassay.

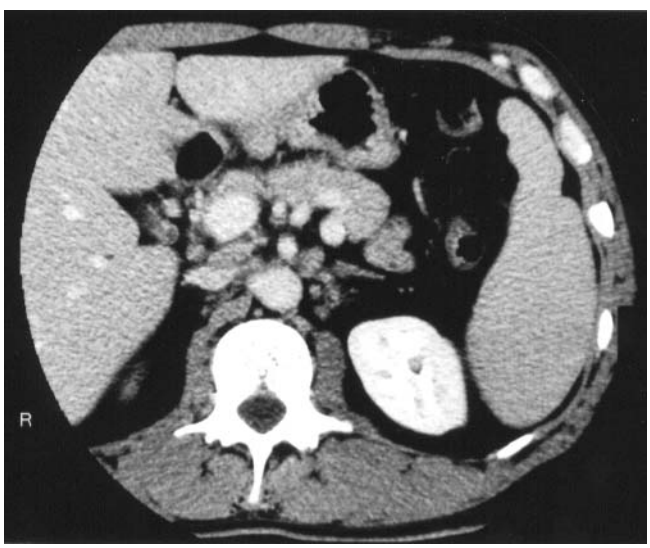

Figure 1. Enhanced computed tomography shows marked portocaval, peripancreatic and para-aortic lymphadenopathy and splenomegaly.

Discussion

Initial diagnosis of IM in older individuals is often difficult, since easily recognized IM findings such as tonsillopharyngitis, cervical lymphadenopathy and absolute lymphocytosis accompanied by atypical lymphocytes and heterophile-antibody may be mild or absent (1, 6) as in the present case. These inconclusive findings can result in delayed appropriate diagnosis or highly disparate diagnosis such as active viral hepatitis, acute cholecystitis, obstructive jaundice, bronchopneumonia or malignant lymphoma (1, 6). The present patient was at first considered to have acute hepatitis of unknown origin or lymphoproliferative disease complicating liver injury, because these symptoms far from satisfied Sumaya's diagnostic criteria for IM (Table 2) (7) until exudative tonsillopharyngitis and cervical lymphadenopathy were detected on the sixth hospital day. In addition, significantly elevated hepatic enzymes, over five-fold the normal range (8), and intra-abdominal lymphadenopathy (9) were not typical of IM. Furthermore, dual positive IgM antibodies to EBV and CMV strongly suggested IM, but led to diagnostic confusion. It has been reported that CMV-induced IM-like illness affects older individuals or occurs

Table 1. Transitions of antibody titer of Epstein-Barr virus (EBV) and cytomegalovirus (CMV). CMV-IgM and CMV-IgG were measured by means of enzyme immunoassay technique, and EBV-VCA-IgM, EBV-CVA-IgG and EBNA-IgG by means of fluorescent antibody technique.

Day after hospitalization	1st	15th	27th	93rd
EBV VCA IgM (<×10)	×40	×20	×40	<×10
EBV VCA IgG (<×10)	×160	×320	×640	×320
EBV EBNA (<×10)	<×10	<×10	<×10	×10
CMV IgM (<0.8)	2.07 (+)	0.85 (+/–)	0.81 (+/–)	0.68 (–)
CMV IgG (<2.0)	<2.0 (–)	<2.0 (–)	<2.0 (–)	<2.0 (–)

Table 2. Sumaya's diagnostic criteria for infectious mononucleosis (7). Diagnosis of infectious mononucleosis can be made, when at least three of the clinical manifestations and hematological findings are concurrently present.

Clinical manifestation:	1. fever
	2. tonsillopharyngitis
	3. cervical adenopathy
	4. hepatomegaly
	5. splenomegaly
Hematological findings:	
	at least 50% or $\geqq$5,000 lymphocytes/μl and
	at least 10% or $\geqq$1,000 atypical lymphocytes/μl

less frequently and in a milder form than in EBV-induced IM (3, 10), but these never become conclusive criteria for differentiation (2). A positive heterophile test certainly is a helpful clue for the identification of EBV-induced IM, but it cannot always be used because of its low prevalence in older (1) or in Japanese people (11). It took as long as three months to establish EBV-induced IM based on seroconversion to EBNA-IgG positivity and reduced CMV-IgM titer conjunction with persistently negative CMV-IgG. This case clearly illustrates the difficulties of the initial diagnosis for older IM patients because of concomitant abnormal humoral immunity as well as inconclusive IM symptoms. It should thus be noted that aged IM patients present atypical physical and laboratory findings such as prolonged fever without apparent cause, less peripheral lymphadenopathy or more elevated hepatic enzymes (1, 3). Although, to our regret, we failed to determine CMV-DNA in the blood and urine, examination by means of amplification of viral nucleic acid (2, 8) is necessary to avoid a delay in diagnosis and the resultant redundant diagnostic procedures or treatments which increase costs (1), when multi-positive viral serologies are encountered in an IM patient.

Several mechanisms of the simultaneous appearance of IgM to CMV and EBV in IM patients have been proposed such as coinfection of EBV and CMV (2, 12), reactivation of latent EBV infection probably due to transient suppression of cellular immunity by CMV (5, 10, 12–15), selective stimulation of CMV-primed memory B cells by EBV antigen or by induced lymphokines with EBV infection (2, 5), polyclonal B cell stimulation by EBV (4, 5, 13), and truly antigenic cross-reactivity among the herpes viruses including EBV and CMV (13, 15). No detection of IgG-EBNA and CMV-IgG at admission serologically eliminates previous exposure to EBV and CMV, thus excluding either viral reactivation or existence of primed B cells. Both the elimination of CMV infection by a transient increase in IgM followed by persistent absence of IgG, and confirmation of EBV infection by seroconversion of IgG-EBNA, suggest the CMV-IgM test result was false positive. Glycine-alanine-rich elements in EBNA-1 protein (2, 13) and glycine-rich motifs within the non-structural CMV protein of pUL44 and pUL55 are widely used as diagnostic antigens (2). Since cross-reaction to major antigenic epitopes between these elements and motifs have been demonstrated to be a cause of false positive IgM-CMV results, it is tempting to speculate that induced EBV-IgM also recognized the CMV protein in the present case. However, careful interpretation of this finding in combination with IgM-EBV, IgM-CMV, IgG-EBNA and IgG-CMV is essential for a diagnosis of a patient with IM symptoms (15), since IgM-CMV induced by CMV infection adversely cross-reacts with EBNA-1 protein (2, 14). Inconsistent false positive rates of IgM-CMV in IM patients ranging from 20.4% (10) to 40.9% (15), depend probably upon the specificity of kits, i.e. the antigenic CMV protein employed.

In contrast to cervical lymphadenopathy or splenomegaly, little has been published about deep-seated, i.e. intra-abdominal, lymphadenopathy in IM (9). In the case of viral or immune-mediated liver disease, enlargement of perihepatic (9, 16), peripancreatic (17) or retroperitoneal (18) lymph nodes represents activity of regional inflammation and/or immune response of the host, such as viral replication within the liver, direct spread of the hepatic inflammatory process, or recruitment of infected lymphocytes and/or macrophages to draining lymph nodes (16, 18). In the case of EBV infection, proliferation of lymphocytes and/or production of immunoglobulin may be added to the definition of lymphadenopathy because of the lymphotropic nature of EBV and its potent B-cell stimulating action (3). In the present case, intra-abdominal lymphadenopathy and hepatic dysfunction completely preceded over tonsillopharyngitis and cervical lymphadenopathy, and the peak values of transaminases were unusually high for IM-related liver injury. In view of these clinical observations, the possibility cannot be entirely excluded that EBV may have replicated in an unusual manner such as in the liver as well as the oropharyngeal epithelium, or that intra-abdominal lymphadenopathy may serve as a marker for activity of hepatic involvement or systemic immunological alterations (18). These changes include enhanced production of immunoglobulin leading to false positive serologies or complication of autoimmune diseases (3). Needless to say, further studies are necessary to clarify the relationship between intra-abdominal lymph nodes and IM manifestations, hepatic involvement and alterations in the immune system. However, more attention should be also directed to intra-abdominal lymph nodes, when splenomegaly or liver injury is evaluated by means of US or CT in IM patients.

References

1) Horwitz CA, Henle W, Henle G, Schapiro R, Borken S, Bundtzen R. Infectious mononucleosis in patients aged 40 to 72 years: report of 27 cases, including 3 without heterophil-antibody responses. Medicine **62**: 256–262, 1983.

2) Lang D, Vornhagen R, Rothe M, Hinderer W, Sonneborn HH, Plachter B. Cross-reactivity of Epstein-Barr virus-specific immunoglobulin M antibodies with cytomegalovirus antigens containing glycine

homopolymers. Clin Diagn Lab Immunol **8**: 747–756, 2001.
3) Cohen JI. Epstein-Barr virus infections, including infectious mononucleosis. in: Harrison's Principles of Internal Medicine. 14th ed. Fauci AS, Braunwald E, Isselbacher KJ, et al, Eds. McGraw-Hill, New York, 1998: 1089–1091.
4) Haukenes G, Viggen B, Boye B, Kalvenes MB, Flø R, Kalland KH. Viral antibodies in infectious mononucleosis. FEMS Immunol Med Microbiol **8**: 219–224, 1994.
5) Linde A, Fridell E, Dahl H, Andersson J, Biberfeld P, Wahren B. Effect of primary Epstein-Barr virus infection on human herpesvirus 6, cytomegalovirus, and measles virus immunoglobulin G titers. J Clin Microbiol **28**: 211–215, 1990.
6) O'Donovan N, Fitzgerald E. Gallbladder wall thickening in infectious mononucleosis: an ominous sign. Postgrad Med J **72**: 299–300, 1996.
7) Sumaya CV, Ench Y. Epstein-Barr virus infectious mononucleosis in children. I. Clinical and general laboratory findings. Pediatrics **75**: 1003–1010, 1985.
8) Markin RS. Manifestations of Epstein-Barr virus-associated disorders in liver. Liver **14**: 1–13, 1994.
9) Tsujimoto T, Ishikawa M, Iioka H, et al. A case of perihepatic lymphadenopathy in infectious mononucleosis: Ultrasonographic observation. Kanzo (Acta Hepatol Jpn) **43**: 221–225, 2002 (in Japanese).
10) Sanchez Echaniz J, Mintegui Raso S, Benito Fernandez J, Corral Carrancejo JM. Mononucleosis syndromes with serology doubly positive to Epstein-Barr virus and cytomegalovirus. An Esp Pediatr **45**: 242–244, 1996 (in Spanish, Abstract in English).
11) Arai E, Katayama I. Infectious mononucleosis. Medicina **23**: 1757–1760, 1986 (in Japanese).
12) Bertram G, Dreiner N, Krueger GR, et al. Frequent double infection with Epstein-Barr virus and human herpesvirus-6 in patients with acute infectious mononucleosis. In Vivo **5**: 271–279, 1991.
13) Rhodes G, Smith RS, Rubin RE, Vaughan J, Horwitz CA. Identical IgM antibodies recognizing a glycine-alanine epitope are induced during acute infection with Epstein-Barr virus and cytomegalovirus. J Clin Lab Anal **4**: 456–464, 1990.
14) Aalto SM, Linnavuori K, Peltola H, et al. Immunoreactivation of Epstein-Barr virus due to cytomegalovirus primary infection. J Med Virol **56**: 186–191, 1998.
15) Deyi YM, Goubau P, Bodéus M. False-positive IgM antibody tests for cytomegalovirus in patients with acute Epstein-Barr virus infection. Eur J Clin Microbiol Infect Dis **19**: 557–560, 2000.
16) Dietrich CF, Lee JH, Herrmann G, et al. Enlargement of perihepatic lymph nodes in relation to liver histology and viremia in patients with chronic hepatitis C. Hepatology **26**: 467–472, 1997 (Erratum in: Hepatology **28**: 1444, 1998).
17) Neri S, Bruno CM, D'Angelo G, Russo S. Peripancreatic lymphadenopathy and extrahepatic immunological manifestations in chronic hepatitis C. Eur J Clin Invest **26**: 665–667, 1996.
18) Gore RM, Vogelzang RL, Nemcek AA Jr. Lymphadenopathy in chronic active hepatitis: CT observations. Am J Roentgenol **151**: 75–78, 1988.