Gamma heavy chain disease (γ-HCD) as iatrogenic immunodeficiency-associated lymphoproliferative disorder: Possible emergent subtype of rheumatoid arthritis-associated γ-HCD

Hiroko Tsunemine,1) Yuriko Zushi,2) Miho Sasaki,2) Yuko Nishikawa,3) Akiyo Tamura,4) Yumi Aoyama,1) Taiichi Kodaka,1) Tomoo Itoh,5) Takayuki Takahashi1)
Here, we report this RA patient with γ-HCD as iatrogenic immunodeficiency-associated LPD following 2 such cases.10,11

CASE REPORT

A 69-year-old female was admitted because of fever, general fatigue, swelling of multiple lymph nodes in the thoracic and abdominal cavities, and pleural and peritoneal effusion in October 2015. She had been treated using MTX for RA since 2001, and using infliximab and adalimumab for Crohn’s disease in 2011. She also underwent right hip and knee replacement surgeries in 2010.

Physically, she was febrile (38.4°C) and the saturation of percutaneous oxygen was as low as 94%. The abdomen was distended and edema of the lower body was noted. Neither superficial lymphadenopathy nor hepatosplenomegaly was observed. On auscultation, mild stridor was heard and vesicular sounds were diminished in the lower bilateral lungs. She did not complain of arthralgia, although mild deformity of extremity joints was observed. CT of the chest and abdomen demonstrated abundant ascites, and multiple small-sized lymphadenopathies at the mediastinum, right pulmonary hilum, abdominal paraaortic, right lilac, and bilateral inguinal regions, suggesting malignant lymphoma. Organized inflammatory lesions in the bilateral lower lobes of the lungs were also noted on chest CT. Therefore, the fever on admission may have been caused by tumoral fever of the suspected lymphoma or organized pneumonia. In addition, the serum concentration of soluble interleukin-2 receptor was markedly increased to 8,640 U/mL (normally 145 to 519 U/mL) (Table 1).

The results of laboratory examinations on admission are shown in Table 1. The hemoglobin concentration was 9.0 g/dL, white blood cell (WBC) count was 7.9×10^9/L with a low percentage of lymphocytes (14.0%), and platelet count was 100×10^9/L. The percentage of reticulocytes was high at 3.5% (normally 0.5 to 1.5%). Hemostat tests revealed PT-INR, APTT, and D-dimer to be 1.93 (normally 0.85 to 1.15), 77.9 seconds (normally 25.0 to 35.0 seconds), and 6.4 μg/mL (normally 0.0 to 1.0 μg/mL), respectively. Although the Coombs tests were negative, serum concentrations of total bilirubin and indirect bilirubin were elevated to 2.7 mg/dL (normally 0.2 to 1.3 mg/dL) and 1.5 mg/dL (normally 0.0 to 0.8 mg/dL), respectively, in addition to low levels of haptoglobin (below 10 mg/dL) and total cholesterol (61 mg/dL), suggesting Coombs-negative autoimmune hemolytic anemia (AIHA) or severe liver dysfunction of unknown cause. The hemoglobin concentration, however, remained around 9.0 g/dL thereafter regardless of suspected AIHA without any specific treatments. Serological tests for human hepatitis B and C viruses were negative, but those for Epstein-Barr virus (EBV) demonstrated a previous infection pattern with negative EBV-early antigen (EA)-IgG test (Table 1), suggesting chronic active EBV infection to be unlikely. On the other hand, the multiplex virus PCR assay detected 2.2×10^2 copies of EBV genome/mL, which later increased to 7.8×10^5 copies/mL.

The serum concentration of IgG was increased to 3,525 mg/dL (normally 870 to 1,700 mg/dL) and an M peak was observed on serum electrophoresis (Figure 1A), revealed to be monoclonal IgG lacking the light chain by serum immunofixation (Figure 1B), indicating γ-HCD. γ-HCD was further confirmed by rocket immunoselection assay (Figure 2). On bone marrow aspiration, the nucleated cell count and granulocyte/erythrocyte ratio were 45×10^9/L and 1.23,

| Table 1. Laboratory findings on admission |
|-------------------------------------------|
| **Hematology** | **Chemistry** | **Serology** |
| WBC 7.9×10^9/L | AST 9 IU/L | T-Chol 61 mg/dL |
| RBC 2,690×10^9/L | ALT 8 IU/L | TG 36 mg/dL |
| Hb 9.0 g/dL | ALP 414 IU/L | Haptoglobin <10 mg/dL |
| MCV 99.6 fl | T.Bil 2.7 mg/dL | Ferritin 874 ng/dL |
| MCH 33.5 pg | I.Bil 1.5 mg/dL | |
| Platelets 100×10^12/L | LDH 141 U/L | CRP 5.77 mg/dL |
| seg 69.5 % | γGTP 54 U/L | IgG 3,525 mg/dL |
| bas 0.5 % | Che 37 U/L | sIL-2R 8,630 U/mL |
| mon 15.5 % | CRE 0.45 mg/dL | RF (-) |
| lym 14.0 % | UA 2.4 mg/dL | EBV-EA-IgG 0.1 |
| atyp. lym 0.5 % | Na 130 mEq/L | EBV-VCA-IgM 0.0 |
| Retic 3.5 % | K 3.9 mEq/L | EBV-VCA-IgG 1.3 |
| Blood coagulation | | EBV-EBNA 1-IgG 1.6 |
| PT(INR) 1.93 | TP 4.9 g/dL | EBV-PCR 2.2×10^5 copies/mL |
| APTT 81.7 Sec | Alb 1.8 g/dL | Direct Coombs (-) |
| D-dimer 6.4 μg/mL | | Indirect Coombs (-) |

Atyp. lym: atypical lymphocytes. Retic: reticulocytes (normally 0.5 to 1.5%). The normal range of PT-INR: 0.85 to 1.15; APTT: 25.0 to 35.0 seconds; D-dimer: 0 to 1.0 μg/mL; IgG: 870 to 1,700 mg/dL; sIL-2R: soluble interleukin-2 receptor (normally 145 to 519 U/mL); RF: rheumatoid factor. EBV-VCA: Epstein-Barr virus viral capsid antigen, EBV-EA: EBV early antigen, EBNA: EBV nuclear antigen. The results were evaluated as negative, faintly positive, and positive when the value was below 0.5, 0.5-0.9, and greater than 1.0, respectively.
respectively. A small number of large immature lymphoid cells were observed; however, analysis of flow cytometry (FCM) was difficult because of the small number of cells. These abnormal lymphocytes were also observed in the peripheral blood (0.8% of WBC), and on FCM, these cells were positive for CD19, but negative for CD20 (dim), CD5, CD10, CD23, sm/cyIg (α, μ, γ, δ), and sm/cyκ and λ light chains. Furthermore, many immature abnormal lymphocytes were observed in the ascites (Figure 3). FCM analysis of these cells yielded identical results to those of the peripheral blood, although cytoplasmic immunoglobulin heavy and light chains were not examined. PCR examination of these lymphocytes demonstrated monoclonal rearrangement of the immunoglobulin heavy chain (IgH) gene, but not the T-cell receptor-γ gene, suggesting neoplastic B cells. Based on these results, a diagnosis of iatrogenic immunodeficiency-associated LPD, which was substantially γ-HCD, was established.

We discontinued oral MTX and administered oral prednisolone (20 mg/day), but not chemotherapy, because of her poor general condition. This treatment reduced the size of generalized lymphadenopathy as evaluated by CT. However, her general condition did not improve and the multiplex virus PCR assay detected multiple virus genomes in the serum (EBV: $7.8 \times 10^5$, cytomegalovirus: $1.7 \times 10^2$, BK virus: $7.5 \times 10^2$ copies/mL), suggesting increased tumor burden of the EBV-related γ-HCD and exacerbated immunodeficiency.

Fig. 1. An M-peak was noted on serum electrophoresis (A). This M-protein was revealed to be monoclonal IgG lacking the light chain by immunofixation (B).

Fig. 2. Rocket immunoselection assay. A precipitation line representing a reaction with the anti-γ-antibody was observed (arrow) even after immuno-precipitation of whole immunoglobulins with anti-κ and -λ antibodies (lane 4).

Fig. 3. Cytospin preparation of ascites. Many large abnormal lymphoid cells were observed. Their nuclear chromatin was fine and some of these abnormal cells were morphologically plasma cell-like (arrows).
She died of pneumonia 2 months after admission. Immunohistological examination of necropsy specimens from the spleen and bone marrow revealed many abnormal lymphocytes positive for IgG, but not for κ and λ light chains (Figure 4). These abnormal lymphocytes were positive for EBV-encoded small RNA (EBER) (Figure 4) but negative for CD20, CD79a, CD138, and Pax5 (data not shown). Only a few abnormal lymphocytes positive for IgG were observed in the necropsied liver.

DISCUSSION

Although γ-HCD is a rare B-cell neoplasm with a wide spectrum of clinical features,1-5 RA-associated γ-HCD is relatively common. To the best of our knowledge, 15 γ-HCD patients with RA as an underlying disease have been reported.2,3,6-11,13-20 Two γ-HCD patients were treated using MTX and corticosteroid10 or MTX alone11 for RA before the onset of LPD, and were later found to have γ-HCD; therefore, γ-HCD in these 2 patients is considered to be iatrogenic immunodeficiency-associated LPD, because this LPD subtype is tentatively defined as that arising in patients with autoimmune diseases with a history of treatment with immunosuppressive agents such as MTX,9 although the causative mechanism of MTX for the development of LPD has not been elucidated. One more γ-HCD patient had been treated using MTX for seronegative RA; however, the patient did not have constitutive symptoms, lymph node swelling, hepatosplenomegaly, tumoral lesions, or bone marrow abnormality at the time of γ-HCD diagnosis.20 Furthermore, the cells that produced the monoclonal γ-heavy chain were not found or identified in this patient. Therefore, γ-HCD in this patient lacked the clinical features of LPD and the diagnosis of iatrogenic immunodeficiency-associated LPD was unlikely. In the remaining 12 γ-HCD patients with RA, MTX or immunosuppressants were not used for RA treatment,2,3,6-8,13-19 although the treatment agent was not described for one patient.7 Therefore, the present case may be the third reported case of γ-HCD as iatrogenic immunodeficiency-associated LPD. Although γ-HCD is currently rare, it may be an emergent disorder because of the increased use of immunosuppressive agents including MTX for the treatment of autoimmune diseases, especially RA, in recent years. In addition, the EBV genome was detected in the serum, and γ-chain-producing cells were positive for EBER in the present patient. EBER was faintly positive in the biopsied lymph node from the similar γ-HCD patient.11 In another γ-HCD patient, the EBV infection status was not described.10 Thus, to our best knowledge, there has been no report of confirmed EBV-related γ-HCD. Immunodeficiency-associated LPD

Fig. 4. Immunopathological examination of necropsied bone marrow. A: many immature large lymphoid cells can be seen (arrows) (HE staining, ×400). B: These abnormal cells were positive for IgG (immunostaining with polyclonal anti-IgG, ×400). C and D: Few cells were positive for κ- and λ-light chains (immunostaining with anti-κ and -λ antibodies, respectively, ×200). E: These abnormal cells were positive for EBV-encoded small RNA (EBER) (×200).
is usually associated with type III EBV latency, which is characterized by full EBV gene expression, including EBV nuclear antigen (EBNA) 2 to 5. The tumor cells in the present patient may have exhibited type III latency because the tumor cells expressed EBER and EBNA 1 was positive on the serological test. However, we did not examine EBNA 2 or latent membrane proteins (LMP) 1 and 2; therefore, the exact type of EBV latency in the present patient was unclear. The relationship between EBV infection and γ-HCD development should be clarified in the future.

The clinical picture of iatrogenic immunodeficiency-associated LPD in the present patient was generalized disease involving the spleen, lymph nodes, bone marrow, peripheral blood, and peritoneum without a bulky mass. The B-cell neoplasm in the present patient, therefore, resembled LPL based on its pattern of tumor infiltration and monoclonal protein production, as described by Wahner-Roedler et al. for a cohort of γ-HCD patients. However, the phenotype of the present case was different from that of typical LPL, as well as iatrogenic immunodeficiency- or EBV-associated LPD, in terms of being negative for CD20, CD138, CD79a, and Pax5. Although the reason for the negativity of these antigens is unclear, they may have been lost through accumulated genetic alteration in the process of tumor development. Alternatively, this phenotype may be characteristic of immunodeficiency- and EBV-associated γ-HCD. Thus, phenotypic and molecular investigations will be required in the future in a cohort of similar patients.

In FCM analysis of abnormal lymphocytes in the peripheral blood, cyIgG was undetectable regardless of the high possibility of γ-chain production by these cells. The negative result may have been due to the monoclonal anti-γ antibody used in FCM analysis. The γ-chain produced in γ-HCD is subsequently truncated, being an incomplete IgG molecule (one-half to three-quarters of the length of the normal γ-chain); therefore, detection of incomplete IgG by the monoclonal antibody may not have been possible. Indeed, in another γ-HCD case, we were able to detect cyIgG using the polyclonal anti-IgG antibody but not the monoclonal antibody in FCM analysis. On immunohistochemistry of a necropsy specimen from the present patient, we successfully detected IgG in abnormal lymphocytes because we used the polyclonal anti-IgG antibody. The presence of many IgG-positive tumor cells in the necropsied bone marrow may reflect cumulative tumor infiltration over 2 months because the marrow aspirate on admission only included a small number of abnormal cells on morphological and FCM evaluation.

In conclusion, we reported a rare case of iatrogenic immunodeficiency-associated γ-HCD, which may be an emergent subtype of γ-HCD. Further phenotypic and molecular investigations are required involving similar cases in the future.

ACKNOWLEDGMENTS

The authors are grateful to Miss Mizue Higashi for her excellent support for manuscript preparation and literature search.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this study.

REFERENCES

1. Franklin EC, Lowenstein J, Bigelow B, Meltzer M. Heavy chain disease-A new disorder of serum γ-globulins. Am J Med. 1964; 37 : 332-350.
2. Esserman EF, Takatsuki K. Clinical and immunochemical studies of four cases of heavy (Hy2) chain disease. Am J Med. 1964; 37 : 351-373.
3. Fermand JP, Brouet JC, Danon F, Seligmann M. Gamma heavy chain “disease”: heterogeneity of the clinicopathologic features. Report of 16 cases and review of the literature. Medicine (Baltimore). 1989; 68 : 321-335.
4. Wahner-Roedler DL, Kyle RA. Heavy chain diseases. Best Pract Res Clin Haematol. 2005 ; 18 : 729-746.
5. Cook IR, Harris NL, Isaacson PG, Jaffe ES. Heavy chain diseases. In: Swerdlow SH, Campo E, Harris NL, et al. (eds) : WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, Lyon, IARC, World Health Organization. 2017 ; pp. 237-240.
6. Wahner-Roedler DL, Witzig TE, Loehrer LL, Kyle RA. Gamma-heavy chain disease: review of 23 cases. Medicine (Baltimore). 2003 ; 82 : 236-250.
7. Bieljauskas S, Tubbs RR, Bacon CM, et al. Gamma heavy-chain disease: defining the spectrum of associated lymphoproliferative disorders through analysis of 13 cases. Am J Surg Pathol. 2012 ; 36 : 534-543.
8. Husby G, Blichfeldt P, Brinch L, et al. Chronic arthritis and gamma heavy chain disease: coincidence or pathogenic link? Scand J Rheumatol. 1998 ; 27 : 257-264.
9. Gaulard P, Svardlow SH, Harris NL, Sundström C, Jaffe ES. Other iatrogenic immunodeficiency-associated lymphoproliferative disorders. In: Swerdlow SH, Campo E, Harris NL, et al. (eds) : WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, Lyon, IARC, World Health Organization. 2017 ; pp. 462-464.
10. Munshi NC, Digumarthy S, Rahemtullah A. Case records of the Massachusetts General Hospital. Case 13-2008: a 46-year-old man with rheumatoid arthritis and lymphadenopathy. N Engl J Med. 2008 ; 358 : 1838-1848.
11. Kiyasu J, Arakawa F, Haji S, et al. Methotrexate-associated lymphoproliferative disorders with angioimmunoblastic T-cell lymphoma-like features accompanied by gamma-heavy chain disease in a patient with rheumatoid arthritis. Pathol Int. 2018 ; 68 : 485-490.
12. Ito K, Shimizu N, Watanabe K, et al. Analysis of viral infection by multiplex polymerase chain reaction assays in patients with liver dysfunction. Intern Med. 2013 ; 52 : 201-211.
13. Zawadzki ZA, Benedek TG, Ein D, Easton JM. Rheumatoid arthritis terminating in heavy-chain disease. Ann Intern Med. 1969 ; 70 : 335-347.
14 Kretschmer RR, Pizzuto J, González Ll J, López M. Heavy chain disease, rheumatoid arthritis and cryoglobulinemia. Clin Immunol Immunopathol. 1974; 2 : 195-215.

15 Gaucher A, Bertrand F, Brouet JC, et al. [Gamma heavy chain disease associated with rheumatoid arthritis. Spontaneous disappearance of the pathologic protein]. Sem Hop. 1977; 53 : 2117-2120 [in French with English abstract].

16 Ockhuizen T, Smit JW, van Leeuwen M. Rheumatoid arthritis associated with transient gamma 2-heavy chain disease. Rheumatol Int. 1983; 3 : 167-172.

17 Stühlinger W, Berek K, Lapin A, Jaschke E, Pastner D. [Gamma 1 heavy chain disease with immune vasculitis and rheumatoid arthritis]. Klin Wochenshr. 1987; 65 : 359-368 [in Germany with English abstract].

18 Dickson JR, Harth M, Bell DA, Komar R, Chodirker WB. Gamma heavy chain disease and rheumatoid arthritis. Semin Arthritis Rheum. 1989; 18 : 247-251.

19 Takatani T, Morita K, Takaoka N, et al. γ Heavy chain disease screening showing a discrepancy between electrophoretic and nephelometric determinations of serum γ globulin concentration. Ann Clin Biochem. 2002; 39 : 531-533.

20 Johannis W, Blommer J, Klatt AR, Renno JH, Wielckens K. Gamma heavy chain disease in a patient with rheumatoid arthritis – a laboratory evaluation. Biochem Med (Zagreb). 2012; 22 : 373-379.

21 Chau CM, Zhang XY, McMahon SB, Lieberman PM. Regulation of Epstein-Barr virus latency type by the chromatin boundary factor CTCF. J Virol. 2006; 80 : 5723-5732.

22 Lin P, Molina TJ, Cook JR, Swerdlow SH. Lymphoplasmacytic lymphoma and other non-marginal zone lymphomas with plasmacytic differentiation. Am J Clin Pathol. 2011; 136 : 195-210.

23 Swerdlow SH, Cook JR, Sohani AR, et al. Lymphoplasmacytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al. (eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, Lyon, IARC, World Health Organization. 2017; pp. 232-235.

24 Dojcinov SD, Venkataraman G, Pittaluga S, et al. Age-related EBV-associated lymphoproliferative disorders in the Western population: a spectrum of reactive lymphoid hyperplasia and lymphoma. Blood. 2011; 117 : 4726-4735.

25 Wörner S, Mueller-Hermelink HK, Voelker HU. Clinicopathologic features of adult EBV-associated B-cell lymphoproliferative disease. Pathol Res Pract. 2018; 214 : 207-212.

26 Zushi Y, Sasaki M, Saitoh T, et al. Gamma-heavy chain monoclonal gammopathy with undetermined significance (MGUS). J Clin Exp Hematop. 2019; 59 : 119-123.