ONCOLOGY LETTERS 11: 4147-4151, 2016

SPANDIDOS PUBLICATIONS

T cell receptor rearrangements in a patient with γ-heavy chain disease: A case report

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Received February 5, 2015; Accepted March 15, 2016

DOI: 10.3892/ol.2016.4515

Abstract. Heavy chain diseases (HCDs) are rare B cell lymphoplasma cell proliferative disorders that are characterized by the production of incomplete monoclonal immunoglobulin (Ig) heavy chains without the associated light chains. γ-HCD (IgG subtype) is a rare subtype, with ~150 cases reported in the literature to date; however, to the best of our knowledge, no reports of T cell receptor (TCR) gene rearrangement in γ-HCD exist in the literature. The present study reports the case of an 81-year-old man with γ-heavy chain disease associated with TCR gene rearrangement, identified in lymph node biopsy and bone marrow aspirate specimens. The present case revealed an alternative manifestation of γ-HCD, which may provide additional biological insights into this rare B cell disorder.

Introduction

Heavy chain diseases (HCDs) are a group of rare, systemic syndromes, generally associated with variants of B cell neoplasms, and are characterized by the production of an abnormal immunoglobulin (Ig) heavy chain that is incapable of binding to light chains. HCDs are named according to the type of heavy chain produced: IgA (α-HCD), IgG (γ-HCD) and IgM (μ-HCD). The incidence of γ-HCD is higher than that of α- but lower than that of μ-HCD (1). The median age at diagnosis is 68 years (range, 42-87 years). Patients with HCDs may present with a large number of constitutional symptoms and may exhibit concomitant autoimmune disease (2). The clinical course of γ-HCD is extremely variable and ranges from an asymptomatic benign, or stable process to a rapidly progressive neoplasm leading to mortality within a few weeks. γ-HCD generally presents as a lymphoproliferative disorder, comprising lymphadenopathies, splenomegaly and constitutional symptoms. Due to the heterogeneity of γ-HCD, the choice of therapy should entirely rely on the underlying disorder and clinicopathologic features. Prognosis is variable and the mean survival time has been reported to be 7.4 years (range, 1 month to >21 years) (3,4). Rare as B cell disorders are, it is even rarer for patients to exhibit HCD-associated T cell disorders, and only a few cases have been reported to date (2,5,6). The aim of the present study was to report a case of γ-HCD presenting with T cell receptor (TCR) gene rearrangements, in order to provide additional biological insight into the nature of γ-HCD.

Case report

An 81-year-old man presented with enlargement of the left submandibular lymph nodes in August 2012 at the Cancer Hospital of the Chinese Academy of Medical Sciences (Beijing, China). The patient had been diagnosed with testicular sarcoma and had undergone orchiectomy 12 years prior to admission. His past medical history additionally included hypertension, coronary atherosclerotic heart disease and coronary stent implantation. Physical and ultrasound examination revealed enlarged submandibular lymph nodes. Computed tomography (CT; Ingenuity Core 128; Philips Medical Systems, Inc., Bothell, WA, USA) scanning confirmed multiple enlarged lymph nodes involving the mediastinum, axilla, diaphragmatic, retroperitoneum, and common iliac, external iliac and inguinal lymph nodes. Deep cervical lymph node biopsy suggested hyperplastic lymphoid tissue, primarily in the T region, along with local neutrophil infiltration. In addition, the biopsy indicated that the cell population was polyclonal. Immunohistochemical staining revealed cluster of differentiation (CD)19^{+}, CD20^{+}, CD21^{+} (normally atrophied follicular dendritic cells only), CD23^{+} (normally follicular dendritic cells), CD2^{+}, CD3^{+}, CD5^{+}, B cell lymphoma (Bcl)-2^{++}, Bcl-6^{-}, cyclin D1 (CCND1)$^{-}$, Ki67^{+} (10%) and scattered large cells expressing CD30. The following monoclonal primary antibodies were used for immunohistochemistry (all purchased from OriGene Technologies; ZSGB-BIO, Beijing, China): Mouse anti-human CD19 (cat. no. TA506236; 1:100); mouse anti-human CD20 (cat. no. TA800394; 1:150) rabbit anti-human CD21 (cat. no. TA327627; 1:100); rabbit anti-human CD23 (cat. no. TA506412; 1:150); mouse anti-human CD2 (cat. no. TA500394; 1:100); mouse

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Key words: γ-heavy chain disease, T cell receptor rearrangement, B cell neoplasm

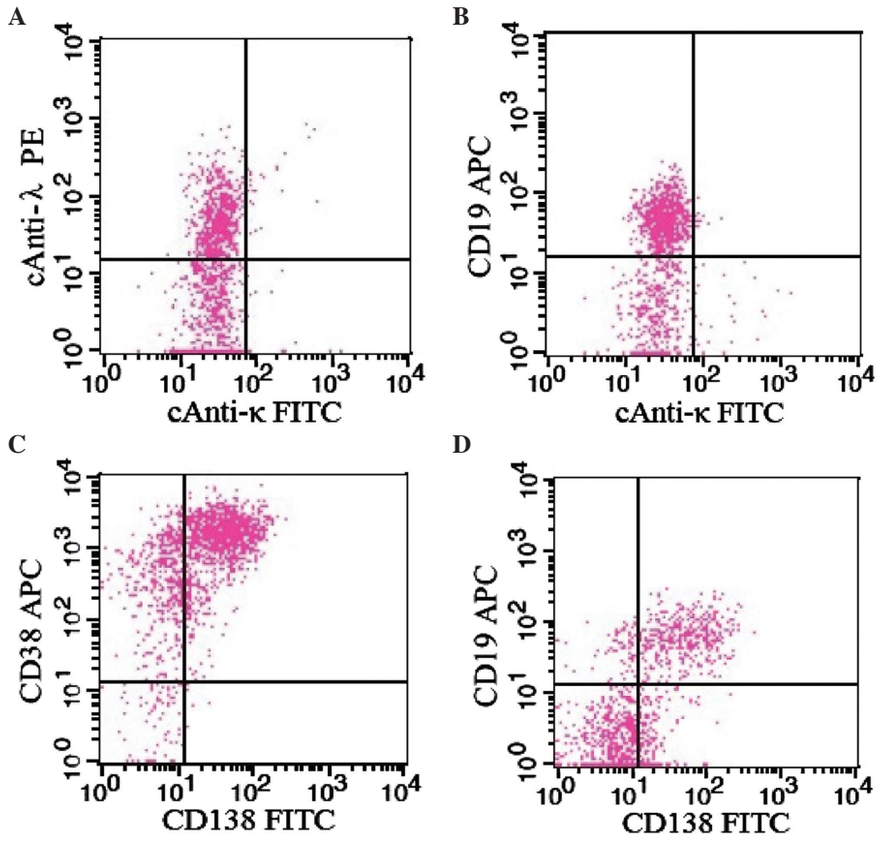

Figure 1. Flow cytometry of bone marrow detected 5.08% atypical lymphoplasmacytic cells, which were positive for cytoplasmic λ, CD19, CD38 and CD138. (A) Atypical lymphoplasmacytic cells demonstrated cytoplasmic light chain λ positivity and cytoplasmic light chain κ negativity. (B) Atypical lymphoplasmacytic cells demonstrated CD19 positivity and cytoplasmic light chain κ negativity. (C) Atypical lymphoplasmacytic cells demonstrated CD38 and CD138 positivity. (D) Atypical lymphoplasmacytic cells demonstrated CD19 and CD138 positivity. The y-axis logarithmic numbers refer to antibodies bound per cell. CD, cluster of differentiation; FITC, fluorescein isothiocyanate; PE, phycoerythrin; APC, allophycocyanin.

anti-human CD3 (cat. no. TA506064; 1:100); mouse anti-human CD5 (cat. no. TA501335; 1:100); mouse anti-human CD30 (cat. no. TA801630; 1:100); mouse anti-human Bcl-2 (cat. no. TA803003; 1:150); mouse anti-human Bcl-6 (cat. no. TA804186; 1:150); rabbit anti-human cyclin D1 (cat. no. ZA-0101; 1:100); and mouse anti-human Ki67 (cat. no. TA802736; 1:100). Horseradish peroxidase conjugated goat anti-mouse and anti-rabbit monoclonal IgG (cat. no. PV-6000; ZSGB-BIO) were used as the secondary antibodies. The PV-9000 kit containing the reagents used for immunohistochemistry was purchased from OriGene Technologies (ZSGB-BIO), and tissue samples were prepared (including staining and sectioning) according to the manufacturer's protocol. PCR analysis (Applied Biosystems™ GeneAmp™ PCR System 9700; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was performed using DNA obtained from the patient's bone marrow and IdentiClone™ IGH/IGK/IGL/TCRB/TCRD/TCRG Gene Clonality Assays (Invivoscribe Technologies, Inc., San Diego, CA, USA) according to the manufacturer's protocol. The PCR cycling conditions were as follow: initial denaturation at 95˚C for 7 min, followed by 35 cycles of 95˚C for 45 sec, 65˚C for 45 sec and 72˚C for 90 sec, followed by a final step at 72˚C for 10 min. This revealed clonal TCR gene rearrangements, positive for TCRβ-B, TCRβ-C and TCRγ-A. Based on these findings, a diagnosis of malignant lymphoma involving the tonsil, nasopharyngeal top wall and right side of the pericardium was suspected. The patient was discharged in September 2012, and subsequently, a chemotherapy regimen of cyclophosphamide (100 mg/24 h), etoposide (100 mg/24 h) and prednisone (30 mg/24 h) was administered for 12 months. Repeated CT scans revealed that the size of the lymph nodes had markedly reduced with treatment.

In December 2013, the patient presented with dyspnea and was referred to the Department of Pneumology (Luhe Hospital, Capital Medical University, Beijing). Upon physical examination, the patient was observed to have an enlarged spleen, as well as bilateral cervical and axillary lymphadenopathies. CT scans of the chest, abdomen and pelvis confirmed splenomegaly and bilateral axillary, hilus pulmonis, mediastinal and retroperitoneal lymph node enlargement, accompanied by polyserous effusions, including bilateral pleural, pericardial, abdominal and pelvic cavity effusions. Thoracocentesis results suggested transudate pleural effusion, which was additionally analyzed by flow cytometry (FCM; FACSCalibur; BD Biosciences, Franklin Lakes, NJ). FCM demonstrated primarily T cells positive for CD38, CD7, CD5, CD4, CD3, CD2 and CD3, and weakly positive for CD10. The $CD4^+CD3^+/CD8^+CD3^+$ ratio was 3.62. The following reagents (all purchased from BD Biosciences) were used for flow cytometry: BD Simultest™ Anti-human κ fluorescein isothiocyanate (FITC)/λ phycoerythrin (PE; dilution, 1:10); monoclonal mouse anti-human

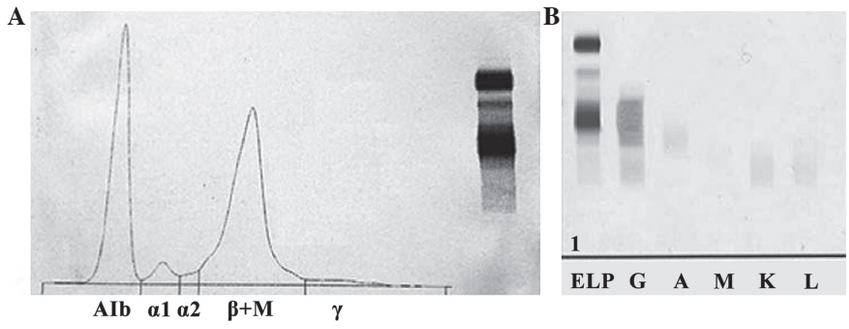

Figure 2. SPEP and IFE. (A) SPEP showing an increased β region, along with decreased albumin levels and γ regions, as well as a loss of separation between the β1 and β2 regions caused by a narrow spike. (B) Serum IFE showing the presence of a monoclonal band in the γ-heavy chain lane without the corresponding band for the light chain lanes. SPEP, serum protein electrophoresis; IFE, immunofixation electrophoresis; ELP, electrophoresis of serum; G, gamma heavy chains; A, albumin; M, μ light chains; K, κ light chains; L,.λ light chains.

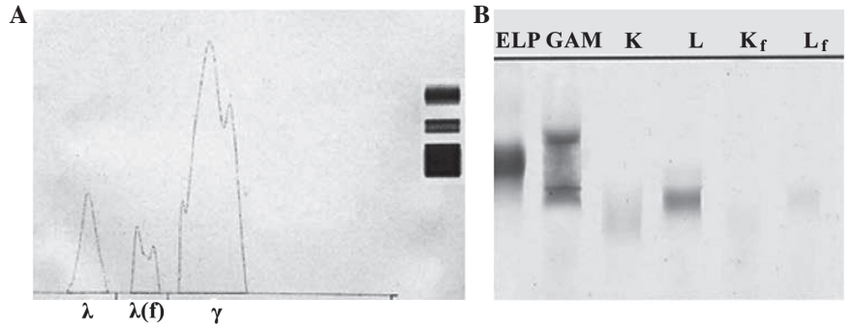

Figure 3. UPEP and urine IFE. (A) UPEP revealing the presence of an unusual spike in the β region. (B) Urine IFE showing an increased immunoglobulin G concentration in the urine and a small amount of associated λ light chain and protein free λ light chain. UPEP, urine protein electrophoresis; IFE, immunofixation electrophoresis; ELP, electrophoresis of serum; GAM, gamma heavy chains; K, κ light chains; L, λ light chains; K_f, free κ light chains; L_f, free λ light chains.

allophycocyanin (APC)-labeled CD19 antibody (catalog no., 340437; dilution, 1:40); monoclonal mouse anti-human FITC-labeled CD138 antibody (catalog no., 347191; dilution, 1:10); monoclonal mouse anti-human APC-labeled CD38 (HB-7) antibody (catalog no., 345807; dilution, 1:40); monoclonal mouse anti-human APC-labeled immunoglobulin (Ig) G_1 (catalog no., 340442; dilution, 1:40); monoclonal mouse anti-human PE-labeled IgG_1 (catalog no., 349043; dilution, 1:10); and monoclonal mouse anti-human FITC-labeled IgG_1 (catalog no., 349041; dilution, 1:10). All samples and reagents were prepared according to the manufacturer's protocol.

Routine laboratory tests revealed leukopenia [white blood cells, $1.61x10^9$/l (normal range, $4\text{-}10x10^9$/l); neutrophils, 92.11% (normal range, 45-77%); lymphocytes, 5.13% (normal range, 20-40%)] accompanied by anemia [hemoglobin, 68 g/l (normal range, 120-160 g/l)] and slight thrombocytopenia [platelet count, $97x10^9$/l (normal range, $100\text{-}300x10^9$/l)], with a normal peripheral blood smear. The erythrocyte sedimentation rate was observed to be increased [25 mm/h (normal range, 0-20 mm/h)]. Total protein plasma concentration was 61.5 g/l (normal range, 64-83 g/l), with albumin levels that were lower than normal [25.2 g/l (normal range, 38-53 g/l)] and globulin levels of 36.2 g/l. Blood urea nitrogen and creatinine levels were within the normal ranges. Skeletal X-rays (DigitalDiagnost Pro; Philips Medical Systems, Inc.) did not reveal any bone lesions.

The patient was subsequently transferred to the Department of Hematology (Luhe Hospital, Capital Medical University, Beijing) with the clinical suspicion of a lymphoproliferative disorder, and more specific tests were performed. Bone marrow aspirate revealed 10% of bone marrow nuclear cells were atypical lymphoplasmacytic cells. Bone marrow FCM confirmed 5.08% of bone marrow nuclear cells were atypical population of lymphoplasmacytic cells, which were $CD19^+$, $CD56^-$, $CD38^+$, $CD138^+$, cytoplasmic IgG^- and cytoplasmic λ^+ (Fig. 1). PCR analysis revealed abnormal clonal Igκ rearrangement, positivity for Vκ-kappa deleting element (Kde) and INTR-Kde, and negativity for Ig heavy locus (IGH) forward 1-3 primers, Vλ-Jλ, DH1-6-JH and DH7-JH. TCRα/β gene rearrangements were observed. The TCRγ gene demonstrated no rearrangements, while the TCRδ gene was weakly positive in the rearrangement assay. Karyotyping revealed a normal male karyotype. Fluorescence *in situ* hybridization testing did not detect any abnormalities in the copy numbers of IGH, 1q21 (a commonly used probe in the prognosis of plasmacyte diseases), tumor protein p53 and retinoblastoma 1, or any IGH-v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog, fibroblast growth factor receptor 3-IGH, IGH-CCND1 and D13S319 (a probe that detects abnormalities at 13q14.3 in chromosomes) fusion genes.

Serum protein electrophoresis (HYDRASYS 2; Sebia Ltd., Camberley, UK) revealed an increased β region, along with decreased albumin levels and γ regions. Furthermore, a loss of separation between the β1 and β2 regions, caused by a narrow spike, was observed (Fig. 2A), A 41.0% (normal range,

60.3-71.4%) 2.52 g/dl; α1 3.8% (normal range, 1.4-2.9%) 0.23 g/dl; α2 1.5% (normal range, 7.2-11.3%) 0.09 g/dl; β +M 51.3% (normal range, 8.1-12.7%) 3.15 g/dl; γ 2.4% (normal range, 8.7-16.0%) 0.15 g/dl. Immunoglobulin quantification revealed an increased level of IgG (74.4 g/l; normal range, 7-16 g/l), accompanied by decreased levels of IgA (0.49 g/l; normal range, 0.7-4 g/l), IgM (0.004 g/l; normal range, 0.4-2.3 g/l), C3 (0.752 g/l; normal range, 0.9-1.8 g/l) and C4 (0.053 g/l; normal range, 0.1-0.4 g/l). Serum immunofixation electrophoresis (IFE; HYDRASYS 2; Sebia Ltd., Camberley, UK) revealed the presence of a monoclonal band in the γ-heavy chain lane without the corresponding band for the light chain lanes (Fig. 2B). Serum free light chains included both decreased free κ (115 mg/dl) and λ (79.6 mg/dl) chains, while the κ/λ ratio was normal (1.44). Urine IFE revealed the presence of an unusual spike in the β region (Fig. 3A), with an increased urine IgG concentration of 0.31 g/dl (total IgG, ~4.65 g/24 h), and a small amount of associated λ light chain and protein-free λ light chain (Fig. 3B); however, the IgG subclasses were all lower than normal [IgG1, <0.43 mg/l (normal range, 4900-11400 mg/l); IgG2, <84.4 mg/l (normal range, 1500-6400 mg/l); IgG3, 75 mg/l (normal range, 200-1100 mg/l); IgG4, 41 mg/l (reference interval, 80-1400 mg/l)].

Based on the aforementioned laboratory findings, the patient was diagnosed with γ-HCD. Dyspnea was treated by thoracic injection of 10 mg dexamethasone, which relieved the symptom to a marked extent. Repeated X-rays revealed that the pleural effusions had disappeared; however, 1 month later, the patient developed acute pancreatitis with increased levels of serum lipase [768 μ/l (normal range, 23-300 μ/l)] and amylase [1,249 μ/l (normal range, 30-110 μ/l)]. As a result, the patient succumbed to persistent high fever and pancytopenia on Feb 25, 2014.

Discussion

Heavy chain diseases (HCDs) are rare B cell lymphoplasma cell proliferative disorders that are characterized by the production of incomplete monoclonal immunoglobulin heavy chains without the associated light chains (3). As the clinical manifestations of HCDs lack specificity, the presence of HCD proteins (a group of abnormal immunoglobulin heavy chains incapable of binding to light chains) is considered the only diagnostic criterion of the disease (1).

HCDs have three main Ig subtypes: IgG, IgM and IgA. Among them, γ-HCD (IgG subtype) is the rarest, with only ~150 cases reported in the literature to date (7). The classification of the lymphoplasmacytic disorder underlying γ-HCD has been controversial (2,4,7,8). The most common subclass of γ-HCD is IgG1, which accounts for 65% of all γ-HCD cases. Other subclasses include IgG3 (27% of all γ-HCD cases), IgG4 (3% of all γ-HCD cases) and IgG2 (2% of all γ-HCD cases) (2). It has been reported that the majority of γ-HCD cases resemble lymphoplasmacytic lymphoma; however, γ-HCD has been reported to be associated with a wide variety of disorders (2,4). In a similar manner to cases of multiple myeloma, γ-HCD cases demonstrated elevated IgG levels corresponding to depressed IgM and IgA (9). The clinical features of γ-HCD include fever, anemia, lymph node enlargement, hepatomegaly, splenomegaly, and in some cases slight leukopenia and thrombocytopenia (2,7). However, the course and prognosis of γ-HCD may vary widely depending on the heterogeneity of the clinicopathological features of the disease (10).

With regard to the present patient, the serum IFE confirmed a monoclonal γ globulin band with no corresponding light chain, therefore suggesting γ-HCD. However, the patient's serum levels of IgG subtypes 1-4 were demonstrated to be lower than normal, which had not been previously reported in the literature to the best of our knowledge (11). As the summation of the IgG subtypes (<2.4 g/l) was markedly lower compared with the total IgG level (74.4 g/l), an antigen excess (also known as excess high-dose hook effect) caused by nephelometric assays was considered to be a potential explanation, as this may have resulted in false negative data (12-14). The discrepancies between the values may additionally have been due to a failure of the IgG subclass antigen to recognize the lost antigenic domain in incomplete heavy chain components. Another discrepancy was observed between the globulin (36.2 g/l) and total immunoglobulin values (74.4 g/l). This could be attributed to the lack of specificity of the biuret test used to detect the protein. In addition, the decreased levels of C3, C4, IgA and IgM may indicate inadequate protein synthesis, leading to infiltration of the immune system in advanced disease.

In addition, both lymph node biopsy and bone marrow aspirate revealed TCR gene rearrangement in the present patient. The bone aspirate additionally revealed Igκ gene rearrangement. Both TCR gene rearrangement in B cell lymphoma (7), and Igκ gene rearrangement in γ-HCD (2) have been reported in the literature; however, to the best of our knowledge, TCR gene arrangement in γ-HCD has not yet been reported. In the present case, the exudative nature of the pleural effusion and immunophenotyping of $CD4^-$ expressing T cell subset indicated strong antigen stimulation or immune dysregulation, which may result in $CD4^+$T lymphocyte activation. Putative extrinsic or intrinsic antigens may trigger B and T cell activation and proliferation, resulting in gene rearrangements in immunoglobulin heavy chains and/or TCR gene clusters (6). As a result of gene deletion of the γ-heavy chain locus, structurally defective IgGs may be formed, leading to γ-HCD (6).

To the best of our knowledge, the present study reports the first case of TCR gene rearrangement in γ-HCD. The present study revealed an alternative manifestation of γ-HCD, which may provide additional biological insights into this rare B cell disorder.

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