Case report

Pulmonary extranodal marginal zone lymphoma that presented with macroglobulinemia and marked plasmacytic cell proliferation carrying the t(14;18)(q32;q21)/MALT1-immunoglobulin heavy-chain fusion gene in pleural fluid

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An 80-year-old man presented with the accumulation of pleural fluid in the right thoracic cavity. Serum electrophoresis revealed an M-component and immunofixation confirmed IgMλ. The level of IgM was 1,526 mg/dL. Imaging studies showed an infiltrative condition of the ipsilateral lung parenchyma. The fluid contained abundant neoplastic cells with the morphological and immunophenotypic features of plasma cells, which expressed IgMλ monoclonal immunoglobulins on the cell surface and in the cytoplasm. The karyotype was 48,XY,+3,add(9)(p13),+12,add(14)(q32),del(16)(q22),−18,+mar, and a series of fluorescence in situ hybridization studies demonstrated that the add(14) chromosome represented der(14)t(14;18)(q32;q21), at which the MALT1-immunoglobulin heavy-chain (IGH) fusion gene was localized. A long-distance polymerase chain reaction amplified the fragment encompassing the two genes, showing that the junction occurred at the J6 segment of IGH and 3.7-kb upstream of the MALT1 breakpoint cluster. We propose that this case represents an extreme form of the plasmacytic differentiation of extranodal marginal zone lymphoma that developed in the lung.

Keywords: macroglobulinemia, extranodal marginal zone lymphoma, plasmacytic differentiation, t(14;18)(q32;q32)/MALT1-IGH fusion gene

INTRODUCTION

Waldenström macroglobulinemia (WM) is a distinct clinicopathological disease entity showing lymphoplasmacytic lymphoma (LPL) in bone marrow and the IgM monoclonal protein in serum (i.e., macroglobulinemia) at any concentration.1,2) WM/LPL is characterized by the proliferation of small lymphocytes admixed with variable numbers of plasma cells and plasmacytoid lymphocytes.2) Patients with WM/LPL present with a number of symptoms attributable to tumor cell proliferation and/or an excess of macroglobulin. However, macroglobulinemia is observed not only in WM/LPL, but also a wide range of B-cell lymphoproliferative disorders, including extranodal marginal zone lymphoma (EMZL) of mucosa-associated lymphoid tissue (MALT lymphoma).3-5) EMZL/MALT lymphoma is composed of morphologically heterogeneous small B cells and the plasmacytic differentiation of lymphoma cells is common.5) Thus, the distinction between WM/LPL and EMZL/MALT lymphoma showing plasmacytic differentiation and associated with the serum IgM monoclonal protein is not necessarily clear, even though >90% cases of the former disease have the MYD88 P265P somatic mutation.2,6)

We herein describe a patient who presented with macroglobulinemia and the accumulation of pleural fluid in the unilateral thoracic cavity. Neoplastic cells in the fluid showed the cytomorphological and immunophenotypic features of plasma cells, indicating the differential diagnosis between WM/LPL and EMZL/MALT lymphoma. Cytogenetic and molecular studies demonstrated a translocation and fusion gene, which was exclusively associated with the latter disease.
CASE REPORT

Case presentation

An 80-year-old man, who had been treated in another hospital for the recurrent accumulation of pleural fluid in the right thoracic cavity for 2 years, was referred to our department because a cytological examination of the fluid suggested lymphoma cells. On examination, breath sounds were decreased in the right hemithorax. There was no surface lymphadenopathy or hepatosplenomegaly. Oxygen saturation was 96% in room air. A chest radiograph showed prominent pleural effusion (Figure 1A). His hemoglobin level was 12.5 g/dL, white blood cell count was 6.95 × 10³/μL, and platelet count was 289 × 10³/μL. Total serum protein was 6.7 g/dL, albumin 3.2 g/dL, globulin 3.5 g/dL, lactate dehydrogenase 307 IU/L, higher than the normal range of 124 to 222 IU/L, creatinine 0.6 mg/dL, and C-reactive protein 2.25 mg/dL. Serum protein electrophoresis revealed an M-component migrating in the γ globulin area and immunofixation confirmed the IgM/λ M protein. No urinary Bence Jones protein was detected. The level of IgG was 723 mg/dL, IgA was 83 mg/dL, and IgM was 1,526 mg/dL (normal range, 33 to 183 mg/dL). Serum free light-chain κ (FLC-κ) was 16.1 mg/L, FLC-λ was 20.5 mg/L, and the κ/λ ratio was 0.79. Soluble interleukin 2 receptor was 3,051 U/mL.

Computed tomography (CT) of the chest revealed the accumulation of pleural fluid in the right thoracic cavity in association with atelectasis of the middle and lower lobes, where the marked accumulation of the tracer was demonstrated by ¹⁸F-fluorodeoxyglucose-positron emission tomography (FDG-PET) combined with CT (Figure 1B), suggesting that the atelectatic lesion contained an infiltrative condition.

Thoracentesis yielded exudative fluid containing abundant neoplastic cells with the morphological features of plasma cells at a concentration of 6.13 × 10³ cells per microliter (Figure 2A). These cells expressed IgM/λ monoclonal immunoglobulins both on the cell surface and in the cytoplasm, and showed a wide range of expression levels of CD20, CD22, CD23, CD38, and CD138. CD19 and CD45 were positive, and CD5, CD10, CD21, CD24, CD56, and surface IgD were negative (Figures 2B and C). The DNA index was 1.06 from normal diploid cells. Bone marrow showed hypercellularity containing CD20-positive plasma cells, and flow cytometry revealed a lymphoplasmacytic population with an identical immunophenotype to that of pleural fluid cells. Tests for the MYD88 L265P somatic mutation were negative (Supplementary Figure S1).

Cytogenetic studies

The G-banding of metaphase spreads obtained from a short-term culture of pleural fluid cells demonstrated numerical and structural abnormalities, including a 14q+ chromosome and uncharacterized small marker chromosome; the karyotype according to the ISCN 2016 was 48,XY,+3,add(9)(p13),+12,add(14)(q32),del(16)(q22),−18,+mar (Figure 3A). Fluorescence in situ hybridization (FISH) of interphase nuclei with the MYC, BCL2, and BCL6 dual-color, break-apart probes detected no rearrangement of these 3 genes, while nuclei carried 3 hybridization signals of BCL2 and BCL6 (Supplementary Figure S2). The hybridization of metaphase cells with the BCL2-IGH dual-color dual-fusion probe showed that the add(14) chromosome was marked by the IGH and BCL2 probes (Figure 3B); however, these probes did not generate a fusion signal (Supplementary Figure S3).

Fig. 1. Imaging studies showing pleural effusion of the right thoracic cavity and an infiltrative condition of the lung parenchyma. (A) Postero-anterior chest X-ray. (B) FDG-PET/CT. The anterior view of a maximum intensity projection image (left) and representative axial images of the thorax (right) are shown. The maximum standardized uptake value of the pulmonary lesion was 17.89.
We then performed hybridization with the \textit{MALT1} dual-color, break-apart probe and found that add(14) was marked by the green-labeled telomeric probe, representing 3'-\textit{MALT1}, while the red-labeled centromeric \textit{MALT1} signal was missing, and, accordingly, interphase nuclei showed the one green and two yellow signal pattern (Figure 3B). The uncharacterized marker chromosome was marked by unrearranged \textit{BCL2} and \textit{MALT1} probes, but not of \textit{IGH}.

These cytogenetic studies indicated that the add(14) chromosome represented der(14)(t(14;18)(q32;q21)), at which the \textit{MALT1}-\textit{IGH} fusion gene was localized, while the reciprocal der(18)(t(14;18)(q32;q21)) was deleted.

\textbf{Amplification and sequencing of the (t(14;18)(q32;q21))/MALT1-IGH junction}

Since the t(14;18)(q32;q21)/\textit{MALT1-IGH} fusion gene was suggested, DNA extracted from pleural fluid cells was subjected to a long-distance polymerase chain reaction (LD-PCR) in order to amplify the junction encompassing the two genes. PCR primers were designed for \textit{MALT1} exon 1 and for the \textit{Eμ} enhancer as well as the \textit{Cμ}, \textit{Cγ}, and \textit{Cα} constant genes of \textit{IGH}; the \textit{MALT1} probe and \textit{IGH} probes were oriented in the opposite direction (Figures 4A and B, Supplementary Table S1). As shown in Figure 4C, we obtained LD-PCR products by the \textit{MALT1}-\textit{Eμ} and \textit{MALT1}-\textit{Cγ} primer combination, indicating \textit{Cμ} to \textit{Cγ} class switching. Nucleotide sequencing of the \textit{MALT1}-\textit{Eμ} product revealed that the junction occurred at the J6 segment of \textit{IGH} and the nucleotide position −5,399 of adenine of the ATG start codon of \textit{MALT1} at the position +1, and a fragment of 9 base pairs (bp) in length was inserted at the junction (Figures 4A and D). As a result of translocation, the \textit{MALT1} and \textit{IGH} \textit{Eμ-Cγ} segments were aligned in the divergent transcriptional orientation. Reverse-transcriptase PCR confirmed the expression of \textit{MALT1} in pleural fluid cells, even though the level was lower than those of \textit{in vitro} cultured lymphoma cell lines (Supplementary Figure S3).

We then applied LD-PCR using the \textit{MALT1}-\textit{Eμ} primer combination to another case with t(14;18) reported previously by us.\footnote{We have applied LD-PCR using the \textit{MALT1}-\textit{Eμ} primer combination to another case with t(14;18) reported previously by us.} The sequences encompassing the \textit{MALT1-IGH} fusion gene were identical to those found in the present case.

Fig. 2. Cytomorphology and immunophenotype of lymphoma cells. (A) May-Giemsa-stained lymphoma cells in pleural fluid, showing a plasmacytic appearance. A few cells had one or more intranuclear inclusions, i.e., Dutcher bodies (right, arrows). (B and C) Flow cytometry of pleural fluid cells using antibodies against the antigens indicated. In B, cells expressed cell surface and cytoplasmic IgM/κ monoclonal immunoglobulins. In C, cells expressed variable levels of B-cell associated antigens and CD38 and CD138, but lacked myelomatous aberrant expression (e.g. CD56).
junction were composed of the J4 segment, a 46-bp insertion fragment, and the upstream sequences of $MALT1$ at the position $-1,738$ of the start codon (Figures 4A and D).

**Treatment course**

The patient was treated with 6 cycles of the DRP (dexamethasone, rituximab, and cyclophosphamide) regimen every 21 days. This led to the resolution of pleural effusion, the disappearance of lymphoma cell invasion in the bone marrow, and accumulation of the tracer within the lung lesion by FDG-PET/CT. However, the serum IgM/$\lambda$. M protein remained detectable by immunofixation and the level of serum IgM remained higher than the normal range.

**DISCUSSION**

We herein described an elderly man who presented with macroglobulinemia and the unilateral accumulation of pleural...
fluid. Lymphoma cells in the fluid showed a plasma cell cytomorphology and expressed CD38 and CD138 antigens, but lacked myelomatous antigen aberrations. Most importantly, cytogenetic studies suggested t(14;18)(q32;q21), leading to the generation of the \textit{MALT1-IGH} fusion gene, which is located at the sub-band 18q21.32 and is oriented from centromere to telomere. The positions of 8 reported breakpoints are indicated by arrows, demonstrating the breakpoint cluster.\cite{16,17,21} Open and closed arrows indicate the breakpoint of the current case and that of Gomyo \textit{et al.},\textsuperscript{7} respectively.

\textbf{Fig. 4.} LD-PCR of the \textit{MALT1-IGH} fusion gene. (A) Genomic structure of the \textit{MALT1} gene, which is located at the sub-band 18q21.32 and is oriented from centromere to telomere. The positions of 8 reported breakpoints are indicated by arrows, demonstrating the breakpoint cluster.\cite{16,17,21} Open and closed arrows indicate the breakpoint of the current case and that of Gomyo \textit{et al.},\textsuperscript{7} respectively. (B) Schematic diagram of LD-PCR of the \textit{MALT1-IGH} junction. The sequences of the primers are described in Supplementary Table S1. (C) Ethidium bromide-stained gel electrophoresis of LD-PCR, showing 7.2- and 11-kb products. (D) Nucleotide sequences of the t(14;18)(q32;q21)/\textit{MALT1-IGH} junction of the present case (top) and those of Gomyo \textit{et al.} (bottom). Vertical lines indicate nucleotide identity. The J6 and J4 segments of \textit{IGH} are boxed and \textit{de novo} nucleotide additions are underlined.

The plasmacytic differentiation of lymphoma cells in association with or without macroglobulinemia may occur in most small B-cell lymphomas, including WM/LPL, chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, mantle cell lymphoma (MCL), and EMZL/MALT lymphoma; however, it varies from being uniformly
present, as in WM/LPL, to very uncommon, as in MCL.\(^5\) Furthermore, the extent of plasmacytic differentiation may vary from minimal to very extensive, resulting in a resemblance to plasmacytoma in extreme cases.\(^4,6,12\) Thus, plasmacytic differentiation does not define any specific type of small B-cell lymphoma. Since the MYD88 L265P somatic mutation is found in most patients with WM/LPL, it has had a significant impact on the differential diagnosis of small B-cell lymphomas; however, this mutation is not entirely specific to WM/LPL and not required for a diagnosis.\(^2,6\) In contrast, translocations involving MALT1, BCL10, FOXP1, and GPR34 are exclusively associated with EMZL/MALT lymphoma;\(^10,11,13,14\) therefore, the detection of these translocations by G-banding, FISH, or appropriate molecular methods is of value for a diagnosis.\(^15\) However, the frequencies of each translocation markedly vary with the primary site of disease and not all tumors carry a translocation; the frequency of t(14;18)(q32;q21) in EMZL/MALT lymphoma arising in the lung ranges between 6 and 10\%.\(^4,11\) Thus, cytogenetic/molecular studies are currently insufficient to effectively discriminate each small B-cell lymphoma category, and the combination of cytomorphic, phenotypic, and sometimes clinical findings is still required in the differential diagnosis of small B-cell lymphomas.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Materials

Supplementary Fig. S1. Allele-specific (AS-) PCR and PCR-RFLP assays detecting MYD88 L265P mutation. For AS-PCR, wild-type (wt) and mutated (mut) alleles were assayed in each sample and the PCR products were electrophoresed side-by-side, and for PCR-RFLP, BsiEI-undigested [BsiEI(−)] and BsiEI-digested [BsiEI(+)] PCR products were electrophoresed side-by-side on a 2% agarose gel. A sample from a case with WM was used as a positive control and a healthy volunteer was used as a negative control.

Supplementary Fig. S2. FISH of interphase nuclei, showing rearrangement of IGH and the lack of rearrangement of BCL2, BCL6, and MYC. Cytospin smear slides or chromosome preparations were hybridized with the dual-color, break-apart (BA) probes for IGH, MYC, BCL6, and BCL2 genes, and the BCL2-IGH dual-color dual-fusion (DF) probe. Hybridization signals are indicated by arrow heads of each color and the pattern of hybridization signals is summarized at the bottom of each picture (R, red signal; G, green signal; Y, yellow signal). The probes were purchased from Abbott Laboratories, Abbott Park, IL, USA.
**METHODS**

**Flow cytometry**

Cells prepared from the pleural fluid were subjected to cell-surface and cytoplasmic antigen analysis using a flow cytometer. Cell distribution was examined in forward versus side scatter cytograms by setting multiple gates, and antigen expression of gated cells was analyzed by multicolor flow cytometry.

**Allele-specific (AS-) PCR and PCR-RFLP for MYD88 L265P mutation**

For AS-PCR, two forward primers were designed to differentiate the wild-type and mutated alleles of MYD88 L265P. To optimize the specificity, an internal mismatch in the third position from the 3’ end was introduced. The sequences of the primers and those of the reverse primer are described in Supplementary Table S1. For PCR-RFLP, PCR amplifying a 726-bp fragment of MYD88 exon 5 was first performed using the primer combination described in Supplementary Table S1 and the amplified fragment was then subjected to BsiEI restriction enzyme digestion for 15 minutes at 60°C. The mutated allele contains a BsiEI site resulting in 448-bp and 278-bp fragments, whereas the wild-type allele does not. All PCR procedures were carried out in a Veriti 96 Well Thermal Cycler (Applied Biosystems, Inc., Forester City, CA, USA).

**Cytogenetic analysis**

Cells prepared from the pleural fluid were incubated overnight under the standard condition, and then cultured in the presence of 0.1 μg/mL colcemid for 2 hr. After harvesting, the cells were treated with hypotonic solution and fixed in methanol:acetic acid (3:1). Chromosomes were banded by trypsin-Giemsa staining and the results of the chromosome analysis were described according to the ISCN 2016.
FISH

The Vysis FISH probe were purchased from Abbott Laboratories, Abbott Park, IL, USA. Denaturing of the chromosome/probe, hybridization, and washing conditions were as recommended by the manufacturer. FISH results were analyzed by a fluorescence microscope (Nikon Corporation, Tokyo, Japan) equipped with DAPI, fluorescein isothiocyanate (FITC), and tetramethylrhodamine B isothiocyanate (TRITC) fluorescence filters, as well as a DAPI/FITC/TRITC triple band-pass filter (Nikon Corporation).

LD-PCR encompassing the MALT1-IGH fusion gene

Genomic DNA was isolated from the pleural fluid cells by means of proteinase K and phenol/chloroform, and subjected to LD-PCR encompassing the t(14;18)(q32;q21)/MALT1-IGH junction. The sequences of the primers are described in Supplementary Table S1. PCR amplification was performed in a Veriti 96 Well Thermal Cycler (Applied Biosystems, Inc., Forester City, CA, USA). PCR products were visualized by ethidium bromide-stained agarose gel electrophoresis, excised from the gel, and cloned into the plasmid (pGEM®-T Easy, Promega). In order to avoid PCR artifacts, three independent cloned DNAs were sequenced by an ABI 310 automated sequencer (Applied Biosystems).

Reverse transcriptase (RT-) PCR

Total cellular RNA was prepared with an RNeasy Mini Kit (QIAGEN, Hilden Germany). First strand cDNA was synthesized from 2 μg of total RNA in the reaction mixture containing random hexamer primers (Roche Applied Science, Penzberg, Germany) and SuperScript® reverse transcriptase (Invitrogen, Carlsbad, CA). PCR primers for RT-PCR and the size of the products were listed in Supplementary Table S1.