Case Report

HIV- and HHV-8-negative Primary Effusion Lymphoma Harboring the Dual Rearrangement of Antigen Receptor Genes and a Translocation of bcl-6

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Primary effusion lymphoma (PEL) is a rare type of lymphoma occurring in HIV-seropositive persons. Since the tumor cells frequently harbor human herpes virus (HHV)-8 DNA, the virus is thought to be causative. We here report an instructive case of PEL in an HIV-seronegative elderly. The neoplastic cells in the fluid showed unusual morphology with multi-lobulated nuclei. They expressed CD19, CD20, CD79a and Smlg/D/M, but not CD3, CD30 or CD23. The IgH gene was clonally rearranged based on the results of Southern blotting and PCR for CDR3. In addition to the IgH clonality, the TCRγ-chain gene was also in the clonal rearrangements. Neither HHV8 nor EBV was detected in the cells. The bcl-6 and IgH chimeric transcripts were evident. Conclusively, our case suggests the following two points: first, the bcl-6/Igh translocation may be associated with the pathogenesis of unusual PEL without HIV or HHV-8; and second, unusual PEL may be an extra-nodal spectrum of diffuse large-cell lymphoma with neoplastic cells harboring the bcl-6/Igh translocation and dual rearrangements of antigen receptor genes.

Keywords: PEL; bcl-6/Igh translocation; HIV; HHV-8

Introduction

Primary effusion lymphoma (PEL), first described by Nador et al., is a rare type of malignant lymphoma occurring exclusively in human immunodeficiency virus (HIV)-seropositive individuals. PEL is characterized as follows: continuous lympomatous effusion in the absence of an identifiable tumor mass, positive status for HIV and Kaposi-associated herpes virus/human herpes virus-8 (KSHV/HHV-8) infection, a distinctive morphology bridging large-cell immunoblastic lymphoma and anaplastic large cell lymphoma with atypia of the nucleus, an aberrant B-cell immunophenotype of CD45⁺, CD30⁺, CD19/20⁺, CD38⁺ and surface membrane immunoglobulin (Smlg)⁺ with clonal immunoglobulin heavy (IgH) gene rearrangements, the presence of Epstein-Barr virus (EBV, HHV-4) DNA within tumor cells and the absence of bcl-2, bcl-6, ras, c-myc and p53 genetic alterations. In particular, HHV-8 has been noted to be a causative agent for the tumorigenesis of PEL.

On the other hand, pleural effusion is well known to present the features of aggressive and advanced lymphomas without an identifiable tumor mass. However, there appears to exist rare cases completely sharing the same clinico-pathological features as PEL. In such cases, it is now controversial whether the infectious status for HIV, HHV-8 and EBV is positive or not, because the original article has stressed the causative relation between the viruses and PEL.

We encountered an HIV-seronegative elderly suffering from chest pain and dyspnea with massive pleural effusion. The cytological and clinical examinations revealed the same morphological and clinical features as PEL, but differed from it in the genetic profiles, such as unusual rearrangements of IgH, TCRγ and bcl-6 genes. Accordingly, we describe here this unique and instructive case among PEL arising in an HIV-seronegative elderly woman, not associated with HHV-4 (EBV) and HHV-8 (KSHV) infection.

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Case report

A 98-year-old woman was admitted to a public hospital because of dysphagia and malnutrition with hemiplegia due to vascular central nervous attack. She suffered from insidious chest pain and dyspnea. On physical examination, pleural effusion on the right side was found, but there was no peripheral lymph-adenopathy or hepatosplenomegaly. Images revealed only massive pleural effusion in the chest X-ray image (Figure 1), but no evidence of detectable tumor mass in the CT scan. The laboratory data at the time of dyspnea was as follows: Hb—10.8 g/dL; WBC—4.5×10⁹/µL with no detectable atypical cells and a lymphocyte fraction of 10.0%; platelets—10×10⁹/µL; total protein—5.8 g/dL; albumin—2.8 g/dL; ALT—20 IU/L; AST—7 IU/L; LDH—309 IU/L; and CRP—1.02 mg/dL. HIV, HCV, HBV and human T-lymphotropic virus type-I (HTLV-I) were serologically negative. The serum level of IgG, IgA and IgM was 670, 60 and 85 mg/dL, respectively. About two liters of pleural fluid was removed twice because of severe dyspnea. The fluid had a bloody color, total proteins of 2.3 g/dL, LDH activity of 1295 IU/L, and negative findings in bacterial culture. The removed fluid was processed to examine the morphological, immunophenotypical and genetic characteristics.

Figure 1. Chest X-ray image at the time of onset, showing the right pleural effusion.

Materials and Methods

Cyto-immunophenotypical studies

The fluid was subjected to an automated hematology analyzer and to direct smears followed by staining with May-Grünwald Giemsa dyes. Immunophenotyping was performed with a flow cytometer, FACScalibur (BD Biosciences, San Jose, CA), following the manufacturer’s instructions, and using commercially available monoclonal antibodies, such as CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD23, CD79a, CD30, HLA-DR, CD25, CD38 and CD45.

Molecular analyses

Genomic DNA and total RNA were extracted from the fluid with a QIamp DNA/RNA Blood Mini Kit (Qiagen, Valencia, CA) following the manufacturer’s instructions.

The status of IgH gene rearrangements was examined using Southern blot hybridization (SBH) and the polymerase chain reaction (PCR) for the complementarity determining region 3 (CDR3) of the IgH hypervariable VDJ region described by us. The clonal rearrangement of the T-cell receptor (TCR)β-chain was also performed with two-step PCR described by Benhattar et al. The presence of HHV-4 and HHV-8 DNA sequences within the cells (total genomic DNAs) obtained from the fluid was examined with PCR using HHV consensus primers and appropriate restrictive enzymes described by us. The genetic analyses were performed using either PCR or RT-PCR according to the methods previously reported by Fan et al. for bcl-2 analysis, Horsman et al. for bcl-6 analysis, and Kawamata et al. and Chen et al. for K-ras analysis, respectively.

Results

Cytological characteristics

The fluid consisted of erythrocytes and mononuclear cells with a cell density of 45,000/µL and a total protein concentration of 2.3 g/dL. Smears showed large (about 15 to 35 µm in diameter) and round lymphoid cells with abundant basophilic cytoplasm and multi-lobulated nuclei, as shown in Figure 2. The chromatin was rich and loose, and the nucleoli were fuzzy. Most neoplastic cells were positive for B-cell associated antigens including SmIgD/MX such as CD19, CD20, CD22 and CD79a, and were negative for T-cell associated antigens of CD3, CD4, CD8, CD5 and CD7. The other immunophenotypic status was positive for HLA-DR, CD38 and CD45, and was negative for CD25, CD10 and TdT.

Figure 2. Morphological features of neoplastic cells from the pleural fluid stained with May-Grünwald Giemsa dyes, showing the polymorphic cells with multi-lobulated nuclei.
Molecular characteristics

To define the linearity and clonality of neoplastic cells, both SBH and PCR analyses were applied. SBH analysis, using the JH probe and restriction enzymes, BamHI and HindIII, produced the rearrangement bands, showing the presence of a predominant B-cell clone, as shown in Figure 3 A. PCR analysis for IgH CDR3 revealed the presence of a monoclonal ampiclon, demonstrating a sharp band in capillary microchip analysis (Figure 3 B). In addition to the clonal IgH gene rearrangement, they exhibited clonal TCRγ rearrangement (Figure 3 C), indicating bi-genotypism. As for the configuration status of oncogenic genes relating to the pathogenesis of lymphoma, PCR analysis gave negative results for bcl-1 and bcl-2 gene rearrangements (Figure 4 A and B) and K-ras mutation at codons 12/13, whereas semi-nested RT-PCR for bcl-6/IgH chimeric transcripts revealed a positive band, as shown in Figure 4 C, indicating that the bcl-6 gene is in the translocation with the IgH gene. Finally, this case was considered to be primary effusion lymphoma with B-phenotypic cells harboring the dual rearrangement of the IgH and TCRγ genes and the translocation of the bcl-6 gene.

Figure 3. Demonstration of the positive IgH clonal rearranged bands in Southern blot hybridization (A), and PCR analysis for IgH CDR3 (B) and the TCRγ clonal band (C). A. Solid arrows and dotted arrows indicate the rearranged bands and the germline bands, respectively. Lanes 1 and 2 correspond to the patient and negative control samples, respectively. M: marker; H: HindIII; H+B: HindIII+BamHI. B and C. The arrows represent the clonal bands. Lanes 1, 2 and 3 correspond to the patient, negative and positive control samples, respectively.

Then, oncogenic viruses relating to human lymphomas were examined using the mononuclear cells in the fluid. Neither the KSHV/HHV-8 nor the EBV/HHV-4 DNA sequence was detected with PCR analysis, as shown in Figure 5. No HTLV-1 was evident using either serology or DNA analysis for the provirus. We unfortunately had no opportunity to perform chromosomal analysis.

Figure 4. Results of the PCR amplicon analyses; lanes 1, 2 and 3 denote the patient, negative-control and positive-control samples, respectively, while M denotes the marker. A. PCR amplicon analysis for the bcl-1/JH rearrangement at the major translocation cluster (MTC) region, showing no rearrangement in bcl-1; duplicate PCR was performed using different primers 1 and 2 to avoid miss-annealing. B. PCR amplicon analysis for the bcl-2/IgH rearrangement at the major breakpoint region (Mbr) and the minor cluster regions (Mcr) regions, showing no rearrangement in bcl-2; duplicate PCR was performed using different primers 1 and 2 to avoid miss-annealing. C. PCR amplicon analysis for the bcl-6/IgH chimeric transcript using cDNA; bcl-6/IgH transcripts were identified.

Figure 5. Results of electrophoretic analyses for fluid cells and peripheral blood using the amplicons digested with BamHI and BstUI. Neither HHV-4 nor HHV-8 DNA sequence was detected. Control-I was HHV-4 negative and HHV-8 negative, while Control-II was HHV-4 positive. ID: indigested; BH: digested with BamHI; BU: digested with BstUI.
Discussion

PEL was originally considered to be a specific subtype of lymphoma, predominantly involving the serous body cavities in HIV-positive immunodeficient individuals with the HIV-8 and EBV imposed-infection. Recently, however, unusual PEL cases clinically mimicking the prototype have been reported increasingly. The unusual issues of such cases in comparison with the prototypic PEL are mainly the absence of HIV infection, including KSHV and EBV, the nature of the aberrant T-cell immunophenotype and the presence of either c-myc or other oncogenic gene alterations.5,11

Thus, our case is also instructive because of seronegativity for HIV and no demonstration of HIV-8 or EBV DNA sequences within the fluid cells, the dual rearrangement of the TCRγ and IgH genes, and the translocation of the bcl-6 gene. The neoplastic cells were morphologically similar to those of PEL, bridging large cell immunoblastic and anaplastic large cell lymphoma with lobulated-nuclei. The immunophenotype is fundamentally of B-cell lineage, but somewhat different from that of the prototypic PEL in terms of Sm1γ, CD19, CD20 and CD30 in our case. This indicates a more mature stage of B-cell ontogeny, rather than that of Sm1γ, CD19, CD20, CD79α and CD30. Genotyping with SBH and PCR for IgH gene rearrangement defined the presence of B-cell clonality, supporting the clonal B-cell lineage. The TCRγ gene was also rearranged based on the PCR result, suggesting abortive processing. This dual rearrangement of antigen receptor genes is well known to be observed in B-acute lymphoblastic leukemia (ALL), which is derived from immature progenitors. Similarly, recent reports have described that about 10% of B-cell lymphomas have genetically dual rearranged antigen receptor genes of TCRγ and IgH. Interestingly, Wakely et al.1 have reported two cases with dual rearrangement of the IgH and TCRB genes among their 5 cases of PEL, suggesting that the dual rearrangement may be common feature in unusual PEL. Unexpectedly, bcl-6 translocation is not evident in their two cases with the dual rearrangement.

As regards genetic alteration in our case, the bcl-6 gene was involved in translocation with the immunoglobulin heavy chain locus. bcl-6 is a zinc finger transcription factor that is highly expressed not only in normal germinal B-cells but also in tumor cells derived from diffuse large cell, Burkitt and follicular lymphomas. This overexpression is considered responsible for the development of lymphoma, because it serves the prevention of differentiation and apoptosis, and the promotion of growth. Indeed, in diffuse large cell lymphoma (DLCL), the translocation of the bcl-6 gene was exhibited in about 50%, and was reported to correlate with clinical presentation at extra-nodal sites including the body cavities,1 suggesting that the bcl-6 translocation is a causative candidate for the pathogenesis of PEL. The prototypic PEL is causatively characterized by the uniform presence of HIV, HHV-8 and EBV. Our case was, however, negative for all three viruses. Although we could not find a causative factor leading to immunodeficiency in our case, the low level of serum Ig and lymphocyte count may indicate the same situation as that of AIDS. Post-transplants also have been reported to develop unusual PEL. Therefore, severe immuno-suppression, related or not to the cause of AIDS, appears to promote the emergence of lymphoproliferative disorders, including PEL.

Of the pathogenesis of HHV-8-negative PEL, Oshimaa et al.15 have suggested, based on the findings of complex genomic abnorrmalities, mainly chromosome 8, in their CGH study, that multi-step carcinogenesis may be involved in HIV/HHV-8 negative cases. Shimazaka et al.16 and Fujisawa et al.17 have presented the pathogenic possibility of c-myc abnormalities, such as c-myc/IgH rearrangement or 8q24 gains, in an unusual PEL case. They reported that a fraction of PEL harbor the Burkitt type c-myc rearrangement, suggesting that lymphomatous effusion is in part one of the extra-nodal symptoms of Burkitt-like lymphoma or DLCL. A half of HHV-8-negative PEL cases had the non-Burkitt type c-myc translocation or the germ line configuration of c-myc with/without oncogene alteration.

Conclusively, the bcl-6/IgH translocation may be deeply associated with the pathogenesis of unusual PEL without HIV or HHV-8, but there remains the possibility that unusual PEL is a spectrum of DLCL with neoplastic cells localized within major body cavities.

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References

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