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Letter to the Editor

Methotrexate-Associated Lymphoproliferative Disorder Mimicking Composite Lymphoma

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Methotrexate (MTX), an inhibitor of dihydrofolate reductase, is administered in patients with autoimmune diseases such as rheumatoid arthritis (RA) to suppress the hyperimmune state [1]. This in turn might induce immunosuppression and provide a basis for the development of lymphoproliferative disorders (LPD): MTX-LPD. MTX-LPD has been included in the immunodeficiency-associated LPD in the recent World Health Organization (WHO) classification for lymphoid neoplasms. A review of more than 100 cases of MTX-LPD reported in the literature revealed the histology to be diffuse large B-cell lymphoma (DLBCL) (35%), Hodgkin's lymphoma (HL) (25%), HL-like lesion (8%), follicular lymphoma (FL) (10%), Burkitt lymphoma (4%), and peripheral T-cell lymphoma (4%) [2].

The occurrence of 2 different and well delineated histologies in a single anatomical site is relatively rare. In such a condition, there are 2 possibilities. One is composite lymphoma with tumor cells derived from a different clone of cells, and the other is transformation of low-grade to high-grade lymphoma. Here, we present a case of MTX-LPD mimicking composite FL and DLBCL. Clonality analysis of laser-captured microdissected specimens revealed the same clonal origin of these lymphomas.

A 61-year-old Japanese woman had presented with polyarthralgia and contracture of the fingers in 1979. Under the diagnosis of RA, she was treated with nonsteroidal anti-inflammatory drugs, steroids, and MTX. MTX (5-7.6 mg/wk) was administered at a cumulative dose of 3842.5 mg. In 1996, she received a diagnosis of mixed connective tissue disease due to the appearance of pulmonary hypertension and autoantibodies (antinuclear and antiribonucleoprotein antibodies). In May 2005, she was admitted to Osaka University Hospital, Osaka, to receive treatment for pulmonary hypertension and right heart failure. Physical examination and computed tomography revealed systemic lymphadenopathy involving the supraclavicular, mediastinal, and para-aortic regions. Splenomegaly but not hepatomegaly was pointed out. Fluorodeoxyglucose positron emission tomography revealed bone marrow invasion. Laboratory studies revealed a leukocyte count of 7870/μL with a normal differential count, hemoglobin 11.2 g/dL, platelet count 7.7×10^4/μL, LDH 548 IU/L, ALP 332 IU/L, C-reactive protein 1.5 mg/dL, and soluble interleukin-2 receptor 1545 U/mL. Two supraclavicular lymph nodes adjoining each other were biopsied. One of the lymph nodes was replaced by a nodular structure with a relatively uniform size (Figure 1A). The small to medium cleaved cells were positive for CD20 (Dako, Carpinteria, CA, USA), CD97a (Dako), and CD10 (Dako). About 90% of these lymphoid cells were positive for bcl-2 (Dako). A diagnosis of follicular lymphoma Grade 2 was made. Histological examination of another lymph node revealed a diffuse proliferation of large lymphoid cells (Figure 1B). These large lymphoid cells were positive for CD20 and CD97a, but negative for CD10. About 60% of these lymphoid cells were positive for bcl-2. In situ hybridization with an Epstein-Barr virus encoded RNA-1 (EBER-1) probe revealed that EBER-1 was negative in both lymph nodes. According to the Ann Arbor schema, the patient had stage IV disease and was included in the high risk group of the international prognostic index. Lymph node swelling remained unchanged after the withdrawal of MTX and 6 courses of rituximab therapy, after which combined chemotherapy (pirarubicin, cyclophosphamide, vincristine, and prednisolone) was started.

To investigate whether the FL and DLBCL were derived from different clones or a single clone, several sections of lymphoma tissues were obtained from both lymph nodes via laser capture microdissection (Leica AS LMD; Leica Microsystems, Wetzlar, Germany). DNA was extracted

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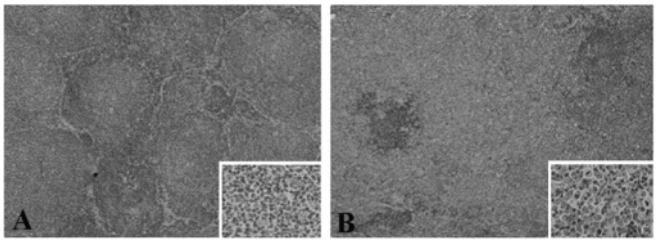

Figure 1. Histology of 2 lymph nodes. A, Follicular lymphoma, Grade 2 in one node (hematoxylin and eosin, original magnification ×40, ×400). B, Diffuse large B-cell lymphoma in another node (hematoxylin and eosin, original magnification ×40, ×400).

from the isolated lymphoma tissues and used for semi-nested polymerase chain reaction (PCR) using immunoglobulin H framework region (Fr) 2A primers. In the semi-nested PCR, primer sets of Fr2A and LJH and primer sets of Fr2A and VLJH were used for round 1 and round 2 reactions, respectively. The sequence of primers was as follows: 5′-TGG(A/G)TCCG(C/A)CAG(G/C)C(T/C)(T/C)C(A/G/T/C)GC-3′ corresponding to the sequence of Fr2A, 5′-TGAGGAGACGGTGACC-3′ corresponding to the sequence of LJH, and 5′-GTGACCAGGGT(A/G/C/T)CCTTGGCCCCAG-3′ corresponding to the sequence of VLJH. A clonal band was detected in both FL and DLBCL specimens, and the size of both bands was identical (Figure 2). The fragments obtained with PCR were inserted into pGEM-T Easy vectors (Promega, Madison, WI, USA), and the source of the amplified fragments was analyzed using an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). To examine the clonality and ongoing somatic mutations, 18 clones were analyzed for each fragment. According to the criteria of Lossos et al [3], a nucleotide substitution mutation observed more than once was considered to be a "confirmed mutation" and not a PCR error. Only confirmed mutations were considered as evidence of ongoing mutations [3]. The sequence of amplified bands from FL showed 4 types of nucleotide variations; typical variation was detected in 12 of 18 clones, and the other 3 variations were detected in 2 clones. These findings indicated the occurrence of ongoing somatic mutations in FL. In contrast, no nucleotide variations were detected in the sequence of the amplified band from DLBCL. The sequence obtained from DLBCL was identical to the typical nucleotide variation found in FL. Lossos et al suggested that the intraclonal heterogeneous sequences in FL could be narrowed down to a single sequence in DLBCL [3], as observed in the present case. These findings clearly showed that the FL and DLBCL in this MTX-LPD were derived from a single clone, ie, occurrence of large cell transformation FL to DLBCL. Because the histologic pictures in the adjoining 2 lymph nodes were quite different with no signs of histologic transition from each other, we cannot deny the possibility of composite lymphomas of different clonal origins without a clonality study. To our knowledge, this is the first report describing MTX-LPD of FL with large cell transformation mimicking composite lymphoma.

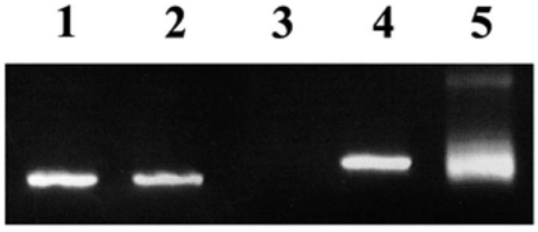

Figure 2. Clonality of lymphoma cells in 2 lymph nodes. The variable-diversity-joining sequences were amplified from DNA of follicular lymphoma (lane 1) and DNA of diffuse large B-cell lymphoma (lane 2). Polymerase chain reaction was also performed with no template (lane 3, negative control) or with DNAs of another case of diffuse large B-cell lymphoma (lane 4, monoclonal band for positive control) and of lymphoid hyperplasia (lane 5, polyclonal bands for positive control).

The relationship between immunosuppression and large cell transformation of low-grade LPD is controversial. Some reports shared that the risk of large cell transformation became high in patients with immunosuppression through administration of the purine analogue fludarabine [4-7]. However, the risk for transformation was not high in immunocompromised patients in other reports [8-10]. Since large cell transformation has not been reported in MTX-LPD, future studies with a large number of MTX-LPD cases will be necessary for the evaluation of transformation risk.

Ott et al reported that the proportion of CD10- or bcl-2–positive lymphoma cells decreased along with the large cell transformation in LPD of immunocompetent patients; 97% and 94% of lymphoma cells were positive for CD10 and bcl-2 in FL, whereas 42% and 75% were positive in large cell transformation of FL [11]. This might explain the different staining patterns for CD10 and bcl-2 between FL and DLBCL in the present case.

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