Clonal Relationship of Extranodal Marginal Zone Lymphomas of Mucosa-Associated Lymphoid Tissue Involving Different Sites

Sergej Konoplev, MD, PhD,* Pei Lin, MD,* Xiaoyan Qiu, MD, PhD, L. Jeffrey Medeiros, MD, and C. Cameron Yin, MD, PhD

Key Words: MALT lymphoma; Multifocal; Clonal relationship; Independent clones; Chronic antigen stimulation

Abstract

Patients with mucosa-associated lymphoid tissue (MALT) lymphoma often have multiple sites of disease at initial diagnosis or during the clinical course. The neoplasms at multiple sites are often presumed to be identical, indicating dissemination or relapse. However, evidence to support this presumption is usually not available. We compared IGH VDJ sequences in 4 patients with 2 sequential sites of MALT lymphoma. The specimen pairs were stomach and nasopharynx, stomach and lung, ocular adnexa and nasopharynx, and ocular adnexa and parotid gland. The median interval between biopsies was 4 months (range, 1-32 months). Monoclonal IGH gene rearrangement was detected in all cases. In 3 patients, the VDJ sequences were distinct; in 1 patient the 2 biopsy specimens shared the same clone. MALT lymphomas involving multiple sites in a patient are usually not clonally related but arise independently, likely due to chronic antigenic stimulation, inducing oligoclonal B-cell proliferations and eventually a dominant B-cell clone.

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is a distinct type of low-grade B-cell lymphoma that represents 7% to 8% of all B-cell non-Hodgkin lymphomas.1 MALT lymphomas can occur at any extranodal site but most often involve stomach, skin, lung, ocular adnexa, and salivary gland. Extensive staging using radiographic imaging and endoscopy of the gastrointestinal tract is often performed in patients with MALT lymphoma to identify all sites of disease. These methods reveal that approximately 25% of patients with gastric MALT lymphoma and 45% of patients with extragastric MALT lymphoma have multiple sites of disease at diagnosis.2,3 It is often presumed that multiple sites of MALT lymphoma at diagnosis represent disease dissemination and that subsequent sites of disease represent persistence or relapse. However, survival of patients with multiple sites of MALT lymphoma is similar to that of patients with unifocal disease in the absence of bone marrow involvement,2 suggesting that multiple sites of extranodal involvement by MALT lymphoma may not necessarily indicate advanced stage of disease. Because treatment decisions are affected by tumor stage or whether a tumor is de novo or relapsed, establishing a clonal relationship for patients with MALT lymphoma involving multiple sites may have clinical implications.

To date, the published literature assessing the clonal relationship of MALT lymphomas involving multiple sites is sparse and mainly focused on gastric MALT lymphoma.4-9 In patients with multiple, discrete lesions of MALT lymphoma involving the same organ, the discrete tumors are often clonally related. For example, Du and colleagues4 reported cases...
of MALT lymphomas involving multiple, distinctive sites within the stomach, and the multiple tumors were all clonally related. By contrast, when a patient has MALT lymphomas involving different organ systems, the data are more mixed. Kawamata and colleagues reported a case of gastric and pulmonary MALT lymphomas with a “near-identical” clonal relationship. Alpen and colleagues, in contrast, reported a case of gastric and pulmonary MALT lymphomas in which the 2 neoplasms were not clonally related.

We studied 4 patients who had 2 MALT lymphomas involving different organ systems sequentially. We used polymerase chain reaction (PCR) methods and sequence analysis to compare rearranged immunoglobulin heavy chain (IGH) VDJ gene segments in each tumor pair. Our data suggest that in patients with sequential MALT lymphomas, the lymphomas are usually not clonally related.

Materials and Methods

Patients

The study was conducted according to an institutional review board–approved laboratory protocol. Informed consent was obtained from the subjects. The files of our hospital from January 1, 2000, to December 31, 2008, were searched for cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease. Inclusion criteria for this study were that patients underwent biopsy of 2 or more anatomic sites involved by MALT lymphoma and that paraffin blocks were available for molecular analysis. For cases of MALT lymphoma involving more than 1 anatomic site at diagnosis or during the course of disease.

Immunophenotypic Analysis

Immunohistochemical stains were performed using formalin-fixed, paraffin-embedded tissue sections; an avidin-biotin-peroxidase complex method; and an automated immunostainer (Ventana-Biotech, Tucson, AZ) as described previously. All tissue sections underwent heat-induced antigen retrieval. The monoclonal antibodies were specific for CD5 (Labvision/NeoMarkers, Montreal, Canada), CD43 (DAKO), and immunoglobulin κ and λ light chains (DAKO).

Sequence Analysis of the IGH VDJ Region

The IGH gene was assessed in all cases using DNA extracted from fixed, paraffin-embedded tissue sections with the QIAamp DNeasy Tissue Kit (Qiagen, Valencia, CA) as described previously. A seminested PCR assay was performed in a 9700 thermal cycler (PE/Applied Biosystems, Foster City, CA). The first round of PCR was performed with primers that anneal to the framework 2 (FR2) region of the variable (VH) segments [5'-TGG(A/G)TCCG(A/C/G) CAG(G/C)(T/C)CC(A/C/G/T)GG-3'] and to a conserved 3' region of the joining region (JH) segments of the IGH gene (5'-AACTGCAAGGAGACGGTGACC-3'). The reaction mix contained 1× PCR reaction buffer, 0.2 mmol/L of deoxynucleoside triphosphates, 0.4 μmol/L of each primer, 2 U of Top Taq DNA polymerase (Qiagen), and 0.5 μg of DNA. The mixture was subjected to 35 cycles of amplification. After incubation at 95°C for 15 minutes, each cycle consisted of denaturation at 94°C for 1.5 minutes, annealing at 59°C for 1.5 minutes, and elongation at 72°C for 3 minutes. The last cycle was followed by a 5-minute elongation step at 72°C. Conditions for the second round of PCR were the same as the first round, except that an FR3 primer [5'-TCGGATCCACGG(T/C)(C/G)TGATTACACTG-3'] was used instead of an FR2 primer. Amplification of a 297-base-pair sequence of the β-actin gene was performed in all cases to confirm that the DNA quality was adequate.

After gel electrophoresis and ethidium bromide staining, the rearranged bands were excised and purified using a QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer’s instructions. The PCR products were cloned into a pGEM-T vector (Promega, Madison, WI), and plasmids were isolated from 2 randomly selected bacterial colonies (DH5α, Invitrogen, Carlsbad, CA) for each case. Plasmid DNA was purified using the GenElute Plasmid Miniprep kit (Sigma Aldrich, St Louis, MO) and sequenced using a T7 primer. DNA sequence analysis using the fluorescence dye terminator method was performed by The University of Texas M. D. Anderson Cancer Center DNA Core Analysis Facility, Houston.

The National Center for Biotechnology Information Basic Local Alignment Search Tool software (BLAST, National Institutes of Health, Bethesda, MD) was used to compare the sequence homology of the PCR products with the germline VH, diversity (DH), and JH sequences and to compare the VDJ sequences within each pair.

Results

Clinical Data

We identified 170 patients with MALT lymphoma diagnosed in our hospital during an 8-year interval. Among them, 11 patients had MALT lymphoma of 2 different anatomic sites and underwent biopsy of these sites. For 4 patients, paraffin blocks of both biopsy specimens of a pair were available for study. The results are shown in Table II.
All patients were women with a median age of 64 years (range, 33-73 years). In case 2, there was a history of Raynaud syndrome. The medical histories in the other 3 cases were unremarkable. The specimen pairs were stomach and nasopharynx, stomach and lung, ocular adnexa and nasopharynx, and ocular adnexa and parotid gland. The median interval between biopsies of the different sites was 4 months (range, 1-32 months). In cases 1 and 2, full staging evaluation, including computed tomography scans of the head, neck, chest, abdomen, and pelvis; endoscopy of the gastrointestinal tract; and unilateral bone marrow biopsy at initial examination showed no evidence of lymphoma other than the biopsied lesions. These cases were therefore considered to be metachronous diseases. Cases 3 and 4 were proven to be synchronous diseases by initial computed tomography scan. One patient (case 2) had received radiation therapy between the 2 biopsies. The other 3 patients were not treated between the 2 biopsies. All patients were treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) after the second biopsy. With a median follow-up of 66 months (range, 18-97 months), 3 patients remained in complete remission, and 1 (case 4) died of secondary acute myeloid leukemia.

Histologically, all 8 neoplasms met the criteria for MALT lymphoma as currently defined. The tumors were composed predominantly of small to medium-sized cells with slightly irregular nuclei, some with abundant, pale (monocytoid) cytoplasm. Scattered large cells were present, but they were not increased and did not form sheets. Occasional lymphoepithelial lesions were noted. The histologic features of the tumors in cases 2 and 4 are illustrated in Image 1A. The bone marrow from case 4 was involved by lymphoma at initial staging evaluation; in the other cases, there was no bone marrow involvement at initial and follow-up studies.

Immunophenotypic studies were performed on all specimens using immunohistochemical methods and fixed, paraffin-embedded tissue sections. The neoplastic cells were of B-cell lineage, positive for CD20 and negative for CD5 in all specimens. Immunoglobulin light chain restriction was detected in some of the 2 patients assessed (cases 1 and 3, all were K restricted). One case (case 2) was stained for CD43, and the neoplastic cells demonstrated aberrant coexpression of CD43. Two pairs (cases 1 and 4) were stained for CD10, and no expression of CD10 was detected in all specimens tested.

Sequence Analysis of the IGH Gene

Monoclonal rearrangement of the IGH gene was detected in both specimens of all 4 tumor pairs [Table 2]. Comparison of the rearranged VDJ sequences in 3 cases (cases 1-3) showed that the tumors in different biopsy specimens had different VDJ sequences, indicating that there was no clonal relationship (Image 1C). In 1 patient (case 4), who had ocular adnexa and parotid gland tumors, the VDJ sequences were identical, indicating clonal identity (Image 1F).

Discussion

Although MALT lymphoma is currently considered a single entity in the 2008 World Health Organization classification of lymphoid neoplasms, there is abundant evidence indicating that MALT lymphomas are extremely heterogeneous. The heterogeneity of this entity is observed on several levels, including suspected etiologic factors, morphologic features, and genetic abnormalities. Infection by Helicobacter pylori is closely linked with gastric MALT lymphoma, whereas other organisms or autoimmune diseases are associated with other MALT lymphomas. Morphologically, lymphoepithelial lesions are very common and prominent in MALT lymphomas of the salivary and thyroid glands but often absent in MALT lymphomas of skin, breast, and the ocular adnexa. A prominent plasma cell component (plasma cells 20% of the infiltrate) is most frequently seen in MALT lymphomas involving the thyroid gland, upper aerodigestive tract, skin, conjunctiva, and breast and is less common in the MALT lymphomas of the stomach, salivary gland, and lung. Four recurrent chromosomal translocations are described in MALT lymphomas, and the abnormalities are not evenly distributed among tumors at different anatomic sites. The t(11;18)(q21;q21), which results in an API2-MALT1 fusion protein, is most common in tumors arising in the lung and stomach. By contrast, the t(14;18) (q32;q21), t(1;14)(p22;q32), and t(3;14)(p14.1;q32) that juxtapose the MALT1, BCL-10, and FOXP1 genes, respectively, with the IGH gene are more common elsewhere. The t(14;18) is most common in MALT lymphomas of the ocular adnexa, skin, and salivary gland. The t(1;14) is rare but tends to occur in MALT lymphomas of the intestine. The t(3;14) tends to be more common in MALT lymphomas of the thyroid gland, ocular adnexa, and skin. These differences in etiologic factors and pathologic and molecular findings suggest that MALT lymphomas arising in different anatomic sites are distinct and that they are likely to be unrelated.
**Image 1** Morphologic features and IgH complementarity-determining region (CDR)3 sequences from representative cases. 

**A** (Case 2), Site 1, stomach (H&E, ×400). **B** (Case 2), Site 2, lung (H&E, ×400). **C** (Case 2), IgH CDR3 sequences from site 1 (upper panel) and site 2 (lower panel) showing that the 2 lymphomas are of different clones. **D** (Case 4), Site 1, ocular adnexa (H&E, ×400). **E** (Case 4), Site 2, parotid gland (H&E, ×400). **F** (Case 4), IgH CDR3 sequences from site 1 (upper panel) and site 2 (lower panel) showing that the 2 lymphomas arise from the same clone.
Many studies have documented that patients with MALT lymphoma have a high frequency of multiple sites of disease. In routine clinical practice, the neoplasms in patients with multiple sites of involvement by MALT lymphomas are staged clinically, with the presumption being that these neoplasms are clonally related. However, the evidence to support this presumption is sparse in the literature and usually absent in the workup of an individual patient. Of interest, survival of patients with MALT lymphoma with multiple sites of disease in the absence of bone marrow involvement is similar to that of patients with unifocal disease, suggesting that multifocal sites in MALT lymphoma may not be equivalent to disease dissemination.

The published literature can be divided into 2 categories: multiple MALT lymphomas involving the same organ system, usually simultaneously, and MALT lymphomas involving different organ systems, simultaneously or sequentially. In patients with multiple MALT lymphomas involving the same organ detected simultaneously, the MALT lymphomas are usually clonally related. By contrast, the results for the few patients with MALT lymphoma involving different organs are mixed. Two groups studied single patients with MALT lymphomas that involved stomach and lung showing a clonal relationship in one patient and different clones in the other patient. In 2 other case reports, authors explored the clonal relationship in one patient and different clones in the other patient. However, further sequence analysis was not performed.

In this study, we analyzed the VDJ sequences of the rearranged IGH gene from paired samples of MALT lymphoma that sequentially involved different organs in 4 patients. In 3 patients, the neoplastic clones were distinct and unrelated. These findings suggest that MALT lymphomas at different sites often arise independently. The explanation for these results is uncertain, but it is reasonable to suggest that chronic antigen stimulation results in polyclonal or oligoclonal expansion of B cells that arise in or home to different MALT organs. Eventually a neoplastic clone evolves in the local microenvironment.

An indirect confirmation of the concept of MALT lymphoma as an oligoclonal B-cell proliferation was obtained by 2 independent studies. Lasota and Miettinen analyzed 3 consecutive biopsy specimens of parotid MALT lymphoma during a 9-year interval using IGH sequencing. While sequences obtained from the first and second specimens were identical, the sequence obtained from the third specimen was distinct. Primers specific to clones identified in the first and second specimens failed to amplify from the third specimen; primers specific to the clone seen in the third specimen failed to amplify from the first specimen but did amplify from the second specimen. The clone detected in the third specimen was estimated to account for fewer than 2% of the major clones in the second specimen. Yamauchi and colleagues22 studied patients with MALT lymphoma of the stomach who underwent gastrectomy. They compared clones in distinct nodules in patients with multifocal involvement as well as in different parts of a single large lesion in patients with unifocal involvement. They detected a monoclonal or biclonal pattern in 49% of lesions and an oligoclonal pattern in 35% of lesions. These findings suggest that gastric MALT lymphomas may initially arise as a polyclonal or oligoclonal B-cell population from which a specific clone can emerge and dominate (become monoclonal) during the course of the disease.

We have also considered the possibility that some patients have a genetic predisposition for developing extranodal oligoclonal B-cell clones and then MALT lymphoma, perhaps accounting for multiple sites of disease. Whereas 1 patient (case 2) had a reported history of Raynaud syndrome, in the 4 patients we have assessed, the patients were not worked up for immune dysfunction or genetic predisposition.

Several groups have performed studies with a similar design analyzing the clonal relationship of tumors arising...
from different anatomic sites in patients with mycosis fungoides.\textsuperscript{23,24} T-cell clones detected at different anatomic sites in patients with mycosis fungoides can be identical, related, or completely distinct.\textsuperscript{23,24} The mechanisms of clonal heterogeneity in patients with mycosis fungoides are unclear. Vega and colleagues\textsuperscript{24} proposed that early lesions of mycosis fungoides may emerge from polyclonal or oligoclonal activation of T cells, and after variable time of antigen stimulation, independent outgrowth of several clones at different sites would occur. It is possible that mechanisms of oligoclonal B-cell expansion in patients with MALT lymphoma are analogous to those in patients with mycosis fungoides.

An identical clone was identified in only 1 patient with MALT lymphomas involving the ocular adnexa and the parotid gland. Taking into consideration the anatomic proximity and shared venous and lymphatic basins of orbital adnexal structure and the parotid gland, it is conceivable that in this particular patient, both neoplasms resulted from lymphatic and/or venous dissemination of the same neoplastic clone. Similarly, the presence of identical clones in MALT lymphomas in the stomach and intestine detected by Du and colleagues\textsuperscript{4} could be explained by shared stomach and intestine venous and lymphatic basins. However, no direct evidence to support this concept is available in the literature.

Our results indicate that sequential MALT lymphomas involving different anatomic sites often demonstrate distinctive clonal IGH gene rearrangements, indicating that these neoplasms are not clonally related. These findings may have implications for our understanding of staging results in patients with MALT lymphoma. Whereas multiple MALT lymphomas within the same organ system and in approximate anatomic location may have resulted from lymphatic and/or venous dissemination of the same neoplastic clone, multiple MALT lymphomas involving different organ systems more often represent different clones, and, therefore, survival of the latter group of patients in the absence of bone marrow involvement may be expected to be similar to that of patients with unifocal disease. Because multifocal diseases are not necessarily an indication of disseminated or advanced disease, patient management should be based on overall assessment of disease status rather than simply on the number of lesions. Sequence analysis of VDJ sequences of the rearranged IGH gene would be helpful in identifying a clonal relationship among different tumors. However, routine sequencing analysis may not be practical in clinical practice.

References


