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LABORATORY INVESTIGATIONS

Clinicopathologic and Immunogenetic Analysis of Mucosa-Associated Lymphoid Tissue Lymphomas Arising in Conjunctiva

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Purpose: To identify mucosa-associated lymphoid tissue (MALT) type lymphoma in conjunctival infiltrates.

Methods: Clinical, histopathologic, immunophenotypic, and immunogenotypic studies were performed on 14 patients with conjunctival lymphoid infiltrates. Surgical biopsy specimens were subjected to histopathologic, immunohistochemical, and gene rearrangement analysis.

Results: Thirteen of the 14 patients (92.9%) met the diagnostic criteria for MALT lymphoma, and the remaining patient showed morphologic features of diffuse, small lymphocytic lymphoma. Genotypic analysis confirmed immunoglobulin heavy chain gene rearrangement in all of the 12 patients on whom the analysis was performed. Two patients with bilateral lesions exhibited identical immunoglobulin rearrangement patterns in each pair of lesions. All patients were alive at the last follow-up (mean: 39.9 months). Nine of the 14 patients were alive without disease, 4 had localized recurrences, and 1 had a residual tumor.

Conclusions: These findings indicate that conjunctival lymphoid infiltrates usually have the features of MALT lymphoma with genotypic B lymphocytic monoclonality and a favorable prognosis. **Jpn J Ophthalmol 1999;43:155–161**

Key Words: Conjuncitival lymphoid infiltrates, extranodal lymphoid proliferation, gene rearrangement analysis, MALT lymphoma, mucosa-associated lymphoid tissue (MALT).

Introduction

Extranodal lymphoid neoplasms often overlap in their clinical and histopathologic features with benign and malignant lymphoproliferative disorders.[1–6] Because extranodal lymphoid tissue lacks the typical structures of lymph nodes, extranodal lymphoid infiltrates do not show characteristic features of nodal malignant lymphomas and show morphologic ambiguity.[2,3] This is one of the reasons why pseudolymphoma (reactive lymphoid hyperplasia) or atypical lymphoid hyperplasia occurs frequently in ocular adnexal lymphoid infiltrates.[1,4,7–9] These diagnostic problems may be resolved in part by advanced immunophenotypic studies of lymphoid cell subpopulations to identify monoclonal lymphocyte infiltration.[4,8] Still, histopathologic and immunophenotypic studies often yield ambiguous results, especially when lymphoid infiltrates are malignant. In recent years, gene rearrangement analysis of immumoglobulin and T cell receptor genes have detected genotypic monoclonality in malignant lymphomas and leuke-

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Table 1. Patient Preoperative Profiles

Patient	Age	Sex	R/L		Allergic Conjunctivitis
1	36	M	L		
2	53	F	R		−
3	51	M	R	Concurrent R-orbit	−
4	40	F	B		−
5	52	F	B		+
6	24	F	B		+
7	70	M	L	Rectum carcinoma, mucinous carcinoma	−
8	39	M	L		−
9	56	F	R	17 y, L-orbit ML	−
10	31	F	B		+
11	46	F	R	7 y, L-conjunctival ML	+
12	61	F	R		−
13	50	F	R	4 y, R-pseudolymphoma concurrent R-parotid gland MALT	−
14	35	F	R		−

*Mean: 46.1 years.

B: bilateral, F: female, L: left, M: male, MALT: mucosa-associated lymphoid tissue lymphoma, ML: malignant lymphoma, R: right.

mias,[10,11] and this method was also applied for ocular adnexal lymphoid infiltrates.[10,12–17] On the other hand, a new diagnostic criterion for B cell malignant lymphoma (mucosa-associated lymphoid tissue [MALT] lymphoma) has been applied to malignant lymphomas arising from extranodal lymphoid neoplasm and has proved effective.[18–20] In this study, we performed histopathologic, immunophenotypic, and immunogenotypic investigations on 14 patients with conjunctival lymphoid infiltrates and found that MALT lymphomas with B lymphocyte monoclonal proliferations were common features in the conjunctival lymphoid infiltrates.

Patients and Methods

Fourteen patients with conjunctival lymphoid infiltrates were examined between 1990 and 1997 at the Sapporo Medical University Hospital. Clinical data were obtained from patients' medical records. Surgical biopsy specimens were processed as follows: for

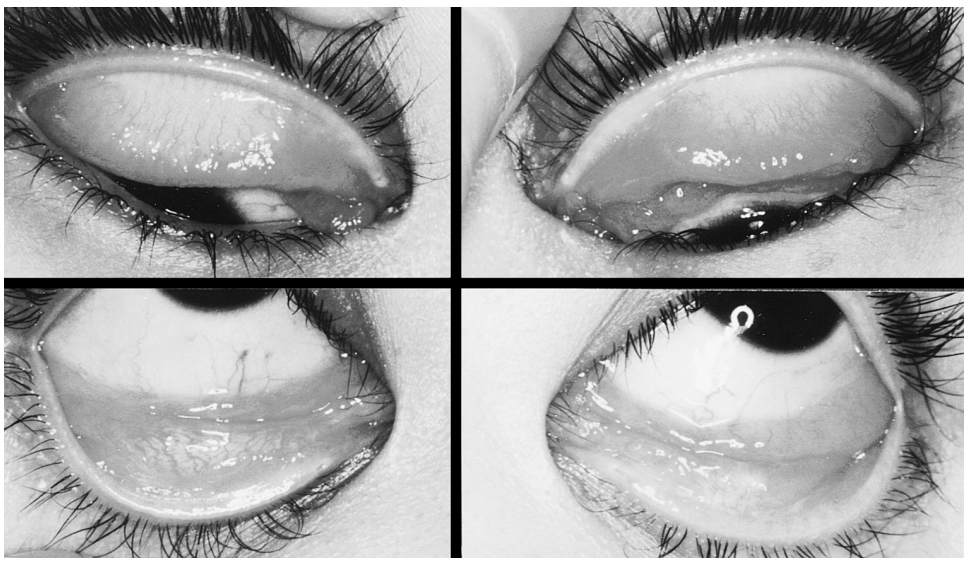

Figure 1. Clinical features of patient 6, showing a bilateral conjunctival tumor with salmon-pink appearance.

Table 2. Histopathologic, Immunophenotypic, Immunogenotypic Results, Treatment, and Outcome for Patients

Patient	CCL	Plasmacytes	Dutcher Bodies	LEL	GC	L26	Light Chain	MT-1 or UCHL-1	JH	Jur β 2	Diagnosis	Treatment	Follow-up Periods (months)*	Last Follow-up	
1	−	+	+	+	−	+	−	+	2R	G	MALT	C, RX	37	NED	
2	+	+	+	+	+	+	−	+	2R	G	MALT	C, RX	10	NED	
3	+	−	−	−	−	+	κ	−	2R	G	MALT	C, RX	60	NED	
4	−	+	−	−	−	+	−	+	2R	G	B-SLL	C, RX	13	NED	
5	+	+	+	+	+	+	κ	+	2R	G	MALT	C	92	AWD	Relapse
6	+	+	+	+	+	+	κ	+	2R	G	MALT	C, RX	56	NED	
7	+	+	+	±	−	+	−	+	R	G	MALT	C	56	NED	
8	+	+	−	+	−	ND	κ	−	2R	G	MALT	C, RX	57	NED	
9	+	+	+	+	−	±	−	+	ND	ND	MALT	C	44	AWD	Relapse
10	+	±	±	+	−	+	−	+	R	G	MALT	C	40	AWD	Relapse
11	+	+	+	+	+	+	−	−	R	G	MALT	C, RX	32	AWD	Relapse
12	+	±	−	−	+	+	−	−	R	G	MALT	−	20	NED	
13	+	±	−	+	+	+	−	+	R	G	MALT	C, RX	26	NED	
14	+	±	−	+	±	+	λ	−	ND	G	MALT	−	16	AWD	Residual tumor

*Mean: 39.9 months.

AWD: alive with disease, B-SLL: B-small lymphocytic lymphoma, C: chemotherapy, CCL: centrocyte-like cells, G: germ line, GC: germinal center–like structure, LEL: lymphoepithelial lesion, MALT: mucosa-associated lymphoid tissue lymphoma, ND: not done, NED: no evidence of disease, R: rearrangement, RX: radiation therapy.

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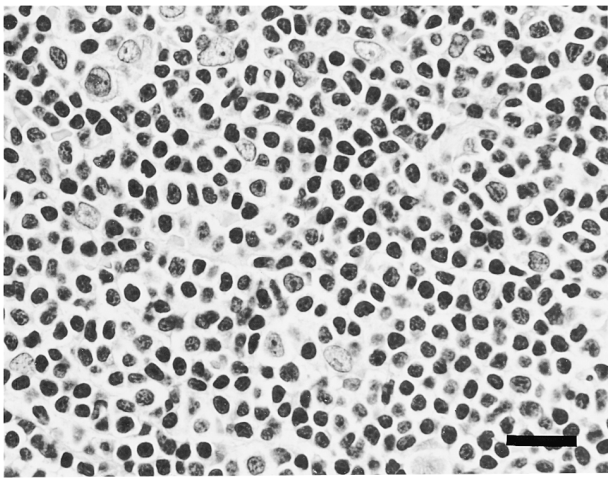

Figure 2. Patient 3: Centrocyte-like cells showing irregular nuclear contour and pale cytoplasm (hematoxylin and eosin, Bar = 20 μm).

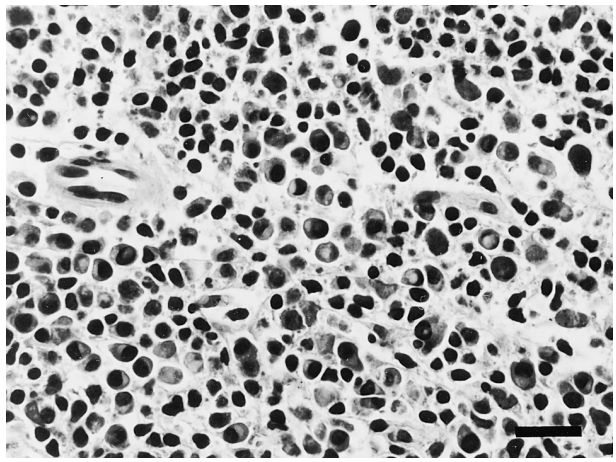

Figure 3. Patient 5: Subconjunctival plasma cells and Dutcher bodies are demonstrated with high magnification (hematoxylin and eosin, Bar = 20 μm).

the histopathologic study, hematoxylin-eosin stain was performed on formalin-fixed, paraffin-embedded specimens. For immunohistochemistry, paraffin-embedded specimens were probed with monoclonal antibodies of L-26 (CD20; Seikagakukogyo, Tokyo, Japan) for pan B lymphocytes and with UCHL-1 (CD45RO; Dako, Carpinteria, CA, USA) or MT-1 (CD-43; Dako) for pan T lymphocytes. In addition, anti–κ chain and anti–λ chain (Beckton Dickinson, Mountain View, CA, USA) were used to detect immunoglobulin light chain restriction. For gene rearrangement analysis, DNA was extracted from frozen biopsy specimens and subjected to Southern blot hybridization. DNA from normal human renal tissue was used as control. DNA samples were digested with EcoRI, HindIII, or BamHI and the digested fragments were electrophoresed and transferred to a nylon membrane. The membrane was then hybridized with DNA probes: namely, the joining region of the immunoglobulin heavy chain gene (JH[11]), and T cell receptor β gene (Jur β2[21]), labeled with [^{32}P]dCTP by a random hexamer method, and then washed and exposed to an x-ray film. Morphologic features described by Isaccson and colleagues[18–20] and in the REAL classification[22] were used for diagnosis of MALT lymphoma.

Results

Clinical Findings

Table 1 provides the clinical features of the patients included in this study. There were 4 men and 10 women, ranging in age from 24 to 70 years (mean: 46.1). Four patients had bilateral conjunctival lesions at initial presentation (patients 4–6, and 10; Figure 1). The lesions were localized in the conjunctiva in 13 patients and the other patient (patient 3) had ipsilateral orbital involvement. Four patients had histories of bilateral allergic conjunctivitis (patients 5, 6, 10, and 11).

Patients 9 and 11 had a history of prior ocular malignant lymphoma; patient 9 had suffered contralateral conjunctival malignant lymphoma 4 years before our initial examination, and patient 11 had contralateral orbital malignant lymphoma 17 years before our initial examination. Patient 13 had a 4-year history of pseudolymphoma in the right conjunctiva. At the time of the initial presentation, a diagnosis of MALT lymphoma in the right conjunctiva concurrent with MALT lymphoma in the right parotid gland was made. Patient 7 had concurrent malignant neoplasms, these being both mucinous carcinoma in the cheek and well-differentiated adenocarcinoma in the rectum.

Pathologic Features

The histopathologic and immunohistochemical findings are summarized in Table 2. Monotonous diffuse lymphocytic infiltration was seen in specimens from all patients. The follicular growth pattern of lymphocytes was not found in any specimen. Infiltrating lymphocytes were mainly small and polymorphic with variable cytologic atypia. Centrocyte-like cells with irregular nuclear contours and pale staining cytoplasm were occasionally seen in 12 of the 14 patients (85.7%) (Figure 2). Plasma cell differentia-

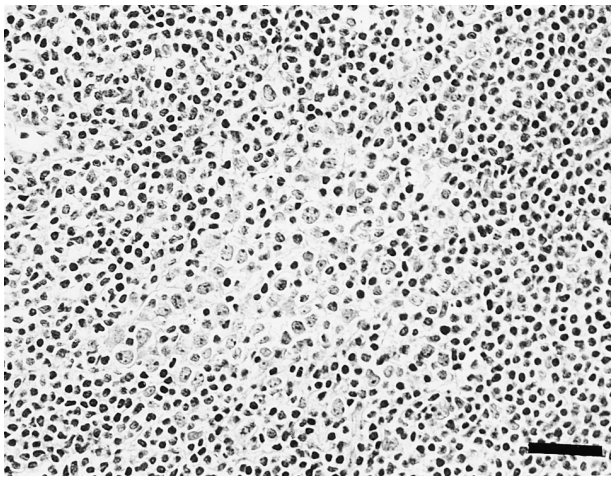

Figure 4. Patient 2: Germinal center-like structure is occasionally seen (hematoxylin and eosin, Bar = 40 μm).

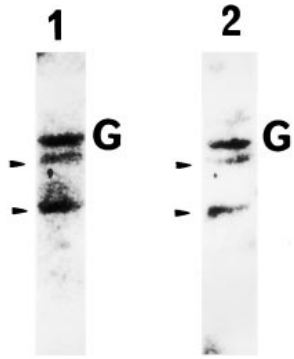

Figure 6. DNA extracted from each site (lane 1 from right eye, lane 2 from left eye) from patient 6 was digested with EcoRI and hybridized to a JH probe. Two identical immunoglobulin heavy chain gene rearrangements (arrowheads) were detected by Southern blot hybridization. (G: germ line.)

tion was found in 13 patients (92.8%). Plasma cells with intranuclear pseudo-inclusions (Dutcher bodies) were seen in seven patients (50%) (Figure 3). Germinal centers with atrophic or residual configurations were in six (42.9%) (Figure 4). In 10 patients (71.4%), the lymphoepithelial lesions, characterized by lymphocytic infiltration into the conjunctival epithelia (Figure 5), were observed. Only patient 3 was classified as having small lymphocytic lymphoma and the remaining 13 patients had features compatible with the morphology of MALT lymphoma.[22]

In the immunohistochemical study of the patients, B lymphocytic infiltration was revealed in 12 by L-26 staining. κ Light chain restriction was revealed in four and λ chain restriction in one. T lymphocytic infiltration was seen in nine by UCHL-1 or MT-1 staining. In the immunogenotypic study, immunoglobulin heavy chain gene rearrangement was found in all 12 patients to whom this method was applied. Identical immunoglobulin heavy chain gene rearrangement bands were demonstrated from each site in two of the four bilateral cases (Figure 6). Immunoglobulin κ chain rearrangement was found in two patients, only one of whom showed immunoglobulin heavy chain gene rearrangement.

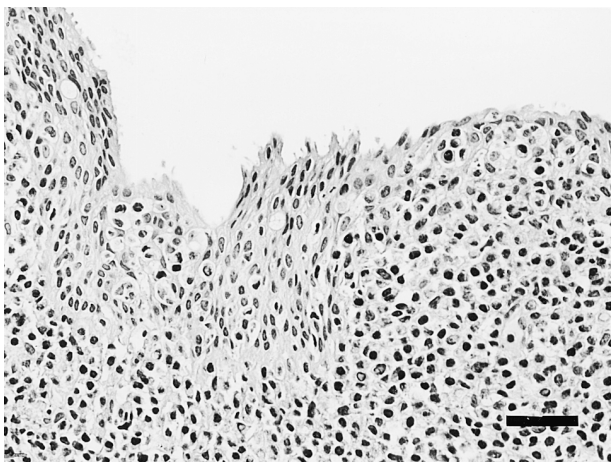

Figure 5. Patient 2: Lymphocytes invade conjunctival epithelium, forming lymphoepithelial lesions (hematoxylin and eosin, Bar = 40 μm).

Treatment and Outcome

Treatment after surgical removal included a combination of radiation and chemotherapy (eight patients), chemotherapy alone (four patients), and surgical excision alone (two patients). The type of chemotherapy varied, but the most common regimen was CHOP (endoxan, adriamycin, vincristine, prednisolone; eight patients). The dose of external beam radiation was 39 Gy. The mean follow-up period was 39.9 months (range: 13–96 months).

At the last follow-up, all patients were alive. Nine (64.3%) of the 14 patients were alive without disease, 4 (28.5%) were alive with localized recurrences in the original site, and one (7.1%) had a residual tumor after surgical excision. There were no patients with systemic dissemination or development of lymphoma in other MALT sites.

Discussion

In our study, all 12 patients in whom rearrangement analysis was performed exhibited immunoglobulin gene rearrangement, indicating that they had

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genotypic monoclonal B lymphocytic proliferation. Recent advances in techniques for the analysis of immunoglobulin and TCR gene rearrangement analysis in lymphomas have eased the task of demonstrating genotypic monoclonality. Gene rearrangement analysis with Southern blot hybridization can detect genotypic monoclonal lymphocytic proliferation even when only 5% of the specimens contain monoclonal lymphocytes.[11] In this study, all 12 patients exhibited genotypic B lymphocytic monoclonality but only 5 patients were disclosed to have distinct immunohistochemical monoclonality (light chain restriction). Genotypic detection of lymphocytic monoclonality may prove to be more sensitive than immunophenotypic examinations.

Among the 14 patients, 13 met the criteria of MALT lymphoma, and only 1 patient was diagnosed as having small lymphocytic lymphoma in the REAL classification.[22] Ocular adnexal malignant lymphoma occurs in about 70% of ocular lymphoid infiltrates; the remainder are classified as reactive lymphoid hyperplasia or borderline lesions (atypical lymphoid hyperplasia).[1,4,5,7,8] It is often difficult to apply a working formulation classification[23] to ocular lymphoid infiltrates, as is the case with other extranodal sites,[2,3] because ocular adnexa lack typical lymph node structure and ocular adnexal lymphoid infiltrates show ambiguous morphology, such as plasma cell differentiation, centrocyte-like cells, and abortive or residual germinal centers.[3,6]

Isaacson and coworkers introduced a new diagnostic criteria, MALT lymphoma, for extranodal B lymphocytic malignant lymphoma.[18–20] The clinicopathologic characteristics of MALT lymphoma are as follows: (1) infiltrating lymphocytes are centrocyte-like cells (marginal zone cells) with irregular nuclei and abundant pale cytoplasm, (2) plasmacytic differentiation with or without Dutcher bodies (intracytoplasmic inclusions), (3) follicular colonization with infiltration of germinal centers by centrocyte-like cells or atrophic germinal center, (4) lymphocyte infiltration to epithelial structures (lymphoepithelial lesion), (5) clinical indolence with rare systemic dissemination, (6) tendency to occur in other MALT sites, and (7) many patients have a history of autoimmune or infectious disease. MALT lymphomas occur not only in mucosal organs (gastrointestinal tract, conjunctiva), but also in nonmucosal epithelia (salivary gland, thyroid, breast) and in nonepithelial tissues (orbit, soft tissue, skin, lung). Most cases of MALT lymphomas appear in the acquired MALT (gastrointestinal tract, salivary gland, thyroid, breast, lung, orbit, conjunctiva, soft tissue, skin), not in the constitutive MALT (tonsil, Peyer's patch, appendix). Acquired MALTs are those in which lymphoid tissues develop secondarily to inflammatory conditions (autoimmune disease or infectious disease). Development of MALT lymphomas in the acquired MALTs are known to be related to preexistent conditions such as Sjögren's syndrome (salivary gland), Hashimoto's disease (thyroid),[18–20] *Helicobacter pylori* gastritis (stomach),[24] and chronic borreliosis (skin).[25] MALT lymphomas in the conjunctiva and orbit have been previously reported.[5,15–17,26] Conjunctival lymphoid tissue is called conjunctival-associated lymphoid tissue (CALT)[27] and it is engaged in B cell–mediated antigen response.[28] It was revealed that MALT is not found in normal human conjunctiva but is acquired during life in certain apparently asymptomatic individuals.[29] In our patients, four had a history of allergic conjunctivitis before receiving a diagnosis of MALT lymphoma. It is not certain whether the allergic conjunctivitis in these patients was a major contributory factor to the development of MALT lymphoma in the conjunctiva or not.

Wotherspoon et al[16] reported seven cases of bilateral conjunctival lymphoid infiltrates without any evidence of extraocular lymphomas. Five of their seven patients exhibited genotypic monoclonal B lymphocyte proliferation and features of MALT lymphomas. Two of those five were revealed to have identical genotypic monoclonality in both eyes. In our 14 patients, 4 patients had bilateral affection, and 2 of those 4 demonstrated identical immunoglobulin heavy chain gene rearrangement at each site. In a malignant neoplasm, a tumor arises from a single cell clone with mutation, and this single clone proliferates and finally metastasizes to other organs. In this respect, we speculate that in the cases with identical gene rearrangement in both sites, the tumor had originated on one side and then spread to the other side (metastasis) rather than developing simultaneously.

Two patients showed previous ocular adnexal lymphoid infiltrates. In retrospective review, one patient had MALT lymphoma in the contralateral conjunctiva 4 years before our initial examination (patient 11) and this case was considered as metastatic. The other patient (patient 9) had contralateral orbital malignant lymphoma (immunoblastic lymphoma) 17 years before our initial examination. At the last follow-up, localized recurrences of conjunctival tumor had occurred in four patients, but all patients are alive without systemic dissemination. This study demonstrated that conjunctival lymphoid infiltrates often displayed histopathologic features of MALT lymphoma with genotypic B lymphocytic monoclonality. Favorable prognosis in these patients is

closely attributed to the clinical behavior of MALT lymphomas.[18–20]

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