Clinical, Histopathological, and Immunogenetic Analysis of Ocular Adnexal Lymphoproliferative Disorders: Characterization of MALT Lymphoma and Reactive Lymphoid Hyperplasia

Tomohiko Mannami, M.D., Tadashi Yoshino, M.D., Ph.D., Koichi Oshima, M.D., Ph.D., Sumie Takase, M.D., Eisaku Kondo, M.D., Ph.D., Nobuya Ohara, M.D., Ph.D., Hideki Nakagawa, M.D., Ph.D., Hiroshi Ohtsuki, M.D., Ph.D., Mine Harada, M.D., Ph.D., Tadaatsu Akagi, M.D., Ph.D.

Second Department of Internal Medicine (TM, MH), Department of Pathology (TY, ST, EK, NO, TA), and Department of Ophthalmology (KO, HN, HO), Okayama University Medical School, Okayama, Japan

Malignant lymphomas and reactive lymphoid hyperplasia (RLH) in the ocular adnexa are sometimes difficult to differentiate morphologically and have often been categorized together as a lymphoproliferative disorder. Immunogenotypic characters of these diseases have not yet been well clarified. This study included 76 cases of ocular adnexal lymphoproliferative disorders. These consisted of 52 cases of malignant lymphoma (43 primary and 9 secondary), 22 of RLH, and 2 borderline cases. There were slightly more male than female subjects. Diagnoses were based on morphology and immunophenotypic characteristics. Clonalities were detected by means of polymerase chain reaction (PCR), and immunoglobulin heavy-chain variable region (VH) genes were sequenced in 10 cases of mucosa-associated lymphoid tissue (MALT) lymphoma. MALT lymphoma constituted 86% (37 cases) of the primary lymphomas. MALT lymphomas were more indolent, more rarely disseminated, and had a lower death rate than the other primary lymphomas. Two patients exhibited coexistence of MALT and diffuse large B-cell lymphoma. The average age of patients with RLH was 5.5 years younger than that of those with MALT lymphoma. One of the cases of RLH later progressed to malignant lymphoma. B-cell clonality was detected by PCR in 57%, 55%, and 0% of primary lymphomas, MALT lymphomas and RLHs, respectively. Sequencing of VH genes revealed that the VH3 family was the most commonly expressed germline VH family (70%) and that DP-63, DP-54 and DP-47 genes were frequently found in the MALT lymphomas examined. PCR analysis was useful for differentiation between MALT lymphoma and RLH. Sequence analysis of VH genes showed that an autoimmune mechanism may be involved in the lymphomagenesis of ocular adnexal MALT lymphoma.

KEY WORDS: Differential diagnosis, Immunogenetic analysis, MALT lymphoma, Ocular adnexa, PCR, Reactive lymphoid hyperplasia.

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Mucosa-associated lymphoid tissue (MALT) lymphomas arise in numerous extranodal sites such as the stomach, salivary gland and thyroid, most of which lack native lymphoid tissue but acquire MALT in close association with chronic inflammation or autoimmune processes. MALT lymphomas appear to have similar clinical, pathological and molecular features regardless of organs of origin (1).

The ocular adnexal region is also a site predisposed to MALT lymphoma. Malignant lymphomas in this site have been subject to diagnostic dilemma because they are composed predominantly of small lymphoma cells often lacking distinct cellular atypia, some of which have an appearance very close to that of small lymphocytes (2). Therefore, it is often difficult to make a differential diagnosis between lymphoma and reactive lymphoid hyperplasia (RLH). In some previous studies, malignant lymphomas and RLH were not clearly distinguished, and they were categorized together as a lymphoproliferative disorder (3). Some investigators have recently reported clinicopathological features of orbital lymphoproliferative disorders along with the identifica-

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Address reprint requests to: Tadaatsu Akagi, M.D., Ph.D., Department of Pathology, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama, 700-8558, Japan; e-mail: akagi@cc.med.okayama-u.ac.jp; fax: 81-86-235-7156.

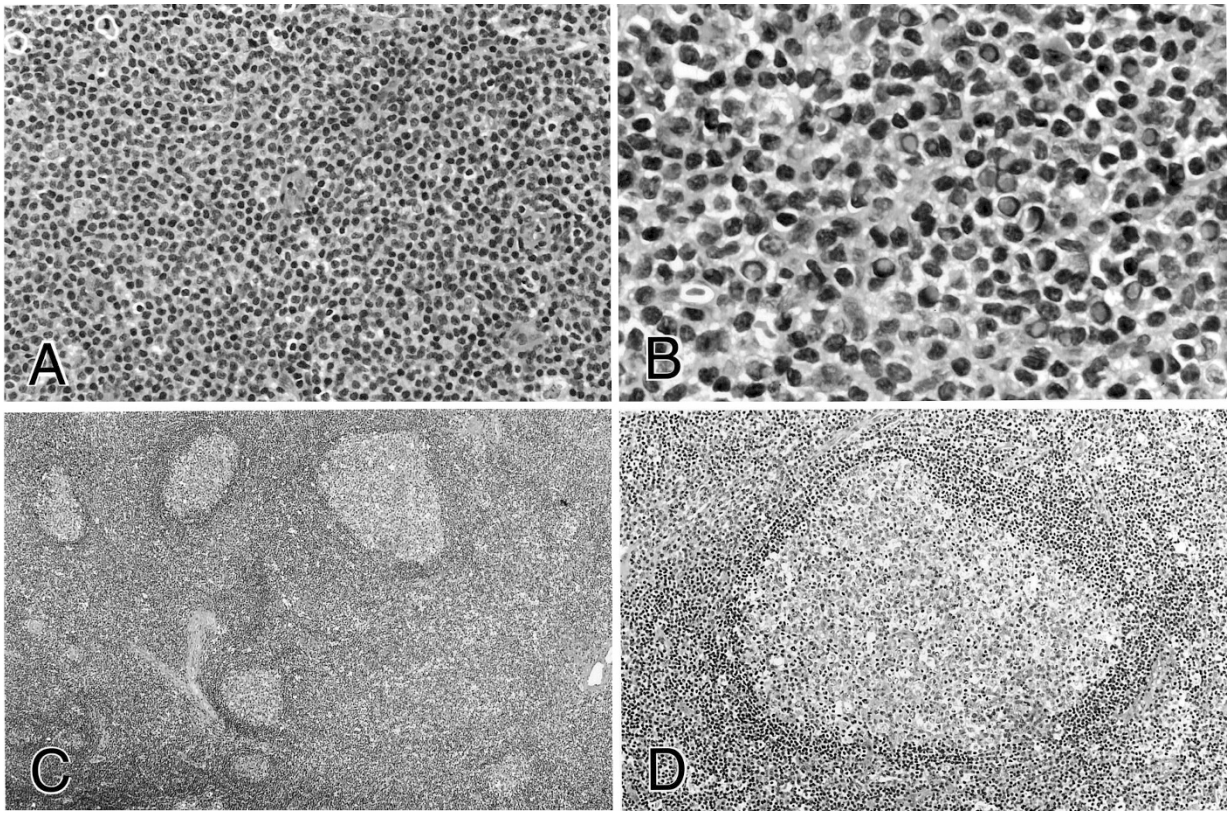

FIGURE 1. Primary MALT lymphoma of the conjuctiva (**A, B**) and reactive lymphoid hyperplasia in the lacrimal gland (**C, D**). **A** (case 17) and **B** (case 25): note monotonous infiltration of centrocyte-like cells with Dutcher bodies. **C** and **D** (case 57): note hyperplastic reactive follicles occupying the lacrimal gland. Lymphocytes in the interfollicular areas show no atypia.

tion of MALT lymphoma (4–7), but few of them referred to the details of RLH.

In the present study, we attempted to characterize clinicopathologic features of ocular adnexal lymphoproliferative disorders, focusing in particular on MALT lymphomas and RLH. We also conducted molecular genetic studies to determine whether MALT lymphomas of this site have characteristics similar to those of other extranodal sites.

MATERIALS AND METHODS

Cases, Diagnostic Criteria, and Clinical Information

Cases involving orbital adnexal region were retrieved from the files between 1989 and 1999 in the Department of Pathology, Okayama University Medical School. All of these cases had hematoxylin and eosin-stained (H & E) sections available for reevaluation, by which 76 consecutive cases of lymphoproliferative disorders were chosen for this study. All patients were Japanese adults. Immunohistochemical staining was performed using an avidin-biotin-peroxidase technique with the following antibodies: monoclonal antibodies (MAbs) to Igκ and Igλ obtained from Novocastra Lab Co. Ltd (Newcastle, U.K.), MAbs to Bcl-2, CD20, CD79a and CD45RO and polyclonal antibodies to CD3 from DAKO Japan (Tokyo, Japan), and MAb to cyclin D1 from Zymed Co. Ltd. (So-SF, CA). Snap-frozen sections were also made in 25 of the cases, and rearrangement of Ig genes was examined by Southern

TABLE 1. Histologic and Immunophenotypic Classification of 76 Ocular Adnexal Lymphoproliferative Disorders

Histologic Category[a]	No. of Cases (%)
Primary ocular adnexal lymphoma	
MALT	37
Diffuse large B cell	5
Mantle cell	1
Total	43 (56)
Secondary ocular adnexal lymphoma	
Diffuse large B cell	3
Follicular	2
Mantle cell	2
Lymphoplasmacytoid	1
Acute lymphoblastic leukemia	1
Total	9 (12)
Atypical lymphoid proliferation	2 (3)
Reactive lymphoid hyperplasia	22 (29)
Total	76 (100)

MALT, mucosa-associated lymphoid tissue.

[a] Diagnosed according to the Revised European-American Classification of Lymphoid Neoplasms. Immunogenotypic analysis by Southern blotting was also applied to 25 cases. Polymerase chain reaction did not change the disease allocation of the individual patients.

TABLE 2. Primary Ocular Adnexal Lymphomas

Case No.	Sex	Age (y)	Diagnosis	Site-bilaterality	Extraorbital involvement	Stage	κ/λ Light-Chain Immunophenotype	Molecular Analysis by Using PCR	Treatment	Follow-Up Period (mo)	Status at Last Follow-Up	Clinical Course
1	F	63	MALT	Orbit, bilateral	None	IEA	λ	—	Observation	120	Alive	
2	F	39	MALT	LG	None	IEA	κ	Clonal	Observation	16	Alive	
3	M	76	MALT	LG	None	IEA	κ	Not clonal	Observation	39	Alive	
4	M	61	MALT	Orbit	None	IEA	λ	Not clonal	Observation	60	Alive	
5	M	56	MALT	Orbit	None	IEA	κ	—	Observation	100	Alive	
6	F	55	MALT	LS	Hard palate	IIEA[a]	λ	Clonal	Rx	98	Alive	Rx to relapse at hard palate (24 mo) and PBSCT to multiple pulmonary relapse (82 mo) achieved CR
7	F	64	MALT	Conjunctiva	None	IEA	κ	Clonal	Observation	87	Alive	
8	M	67	MALT	Conjunctiva, bilateral	None	IEA	κ	Not clonal	Rx	64	Alive	
9	M	67	MALT	Orbit	None	IEA	κ	Clonal	Rx, Cx	87	Alive	
10	F	64	MALT	Conjunctiva	None	IEA	κ	Not clonal	Rx	84	Alive	
11	F	67	MALT	Orbit	None (Bence-Jones protein +)	IEA	λ	Not clonal	Rx	18	Alive	
12	M	71	MALT	Orbit	None	IEA	κ	Not clonal	Rx	12	Alive	
13	M	56	MALT	Orbit	None	IEA	κ	Clonal	Rx	7	Alive	
14	M	68	MALT	Orbit	None	IEA	κ	Not clonal	Rx	6	Alive	
15	M	60	MALT	Orbit	None	IEA	λ	—	Rx	69	Alive	
16	F	86	MALT with DL	LG	None	IEA	Mixed	Not clonal	Rx	11	Alive	Rx was conducted to relapses in auricular node and precordial skin, 4 mo
17	F	27	MALT	Conjunctiva	None	IEA	κ	Clonal	Rx	71	Alive	
18	F	57	MALT	LG	None	IEA	κ	—	Observation	59	Alive	
19	F	57	MALT	Conjunctiva, bilateral	None	IEA	κ	Clonal	Observation	26	Alive	
20	M	67	MALT	Orbit	None	IEA	Mixed	Not clonal	Observation	50	Alive	
21	M	61	MALT	Orbit	None	IEA	κ	Clonal	Observation	20	Alive	
22	M	53	MALT	Conjunctiva	None	IEA		Clonal	Rx	33	Alive	
23	M	79	MALT	Orbit	None	IEA	κ	Clonal	Rx	35	Alive	
24	M	72	MALT	Conjunctiva	None	IEA	Mixed	Clonal	Rx	30	Alive	
25	M	53	MALT	Conjunctiva, bilateral	None	IEA	κ	Clonal	Observation	27	Alive	
26	M	31	MALT	LG	None	IEA	λ	Clonal	—	—	Lost to follow-up	
27	M	67	MALT	Orbit	None	IEA	Mixed	Clonal	Rx	30	Alive	
28	F	44	MALT	Conjunctiva	None	IEA	Mixed	Clonal	Rx	27	Alive	
29	F	23	MALT with DL	Conjunctiva, bilateral	None	IEA	κ	—	Rx	25	Alive	
30	M	78	MALT	Conjunctiva	None	IEA	λ	Clonal	Rx	24	Alive	
31	F	59	MALT	Conjunctiva	None	IEA	κ	Not clonal	Observation	15	Alive	
32	M	80	MALT	Orbit	None	IEA	Mixed	Not clonal	Rx	5	Alive	
33	F	60	MALT	Conjunctiva, bilateral	None	IEA	Mixed	Not clonal	Rx	13	Alive	
34	M	79	MALT	Orbit	None	IEA	κ	Clonal	—	—	Lost to follow-up	
35	F	29	MALT	Conjunctiva	None	IEA	κ	—	Rx	6	Alive	
36	F	85	MALT	Orbit	None	IEA	λ	Not clonal	Rx	7	Alive	
37	M	53	MALT	Orbit	None	IEA	κ	Not clonal	Rx	8	Alive	
38	F	67	DL	LG, bilateral	Systemic surface nodes	IIIEA[b]	κ	Clonal	Cx	111	Alive	Relapse in stomach, 111 mo
39	F	64	DL	Orbit	None	IEA	λ	Clonal	Cx	35	DWD	Dissemination in medulla oblongata, 31 mo
40	M	74	DL	LG	None	IEA	κ	Not clonal	Observation	20	Died NED	Initial treatment was rejected; Cx to disseminated disease (auricular node) achieved CR, 13 mo
41	M	75	DL	Conjunctiva	None	IEA	Mixed	Clonal	Cx	30	Alive	Dissemination in mediastinal and abdominal nodes, 1 mo
42	M	46	DL	LG	None	IEA		Not clonal	Rx	18	Alive	
43	M	69	Mantle cell	Orbit, bilateral	Systemic nodes	IIIEA[c]	(Cyclin D1 +)[d]	Clonal	Cx	37	DWD	Near CR durated for 5 mo; systemic relapses, 17 mo; leukemic change, 35 mo

F, female; M, male; DL, diffuse large B-cell lymphoma; MALT, MALT lymphoma; LG, lacrimal gland; LS, lacrimal sac; Cx, chemotherapy; Rx, radiotherapy; DWD, died with disease; NED, no evidence of disease; CR, complete remission; PBSCT, peripheral blood stem cell transplantation.

[a,b,c] Patient of more than stage I was classified as primary lymphoma because

[a] Chief complaint, lacrimation and palpable tumor, preceded palatal swelling by >12 mo. The former tumor, about 20 mm in diameter, was bigger than the latter at presentation.

[b] Periorbital swelling had lasted for 12 mo, and the orbital tumor was the biggest at presentation.

[c] Conjunctival hyperemia and periorbital swelling lasted for 5 mo. Evaluation at other institutes, 2 and 4 mo before presentation, did not detect any lymphadenopathy.

[d] By immunohistochemistry.

TABLE 3. Secondary or Concurrent Ocular Adnexal Lymphomas

Case No.	Sex	Age (y)	Diagnosis	Site/Bilaterality	Primary Site/Interval to Orbital ML (mo)	Extraorbital Involvement	Immunophenotype	Molecular Analysis by Using PCR	Treatment	Follow-Up Period (mo)	Status at Last Follow-Up
44	F	45	DL	LG	Concurrent	Liver, spleen, bones, BM		Clonal	Cx	7	DWD
45	M	75	DL	Orbit	Concurrent	Ethmoid sinus		—	—	—	Lost to follow-up
46	F	65	DL	Orbit	Systemic nodes (29)	None		—	Cx, Rx	11	DWD
47	F	54	FL	LG	Systemic nodes (117)	None	Bcl-2+	Not clonal	Cx, Rx	49	Alive
48	F	46	FL	Orbit	Palatine tonsil (19)	Cervical node	Bcl-2+	Clonal	Cx	32	Alive
49	M	68	Mantle cell	Conjunctiva, bilateral	Concurrent	Systemic nodes	Cyclin D1 +	—	Cx, Rx	32	DWD
50	M	75	Mantle cell	Orbit	Systemic nodes (53)	Systemic nodes	Cyclin D1 +	—	Rx	2	Alive
51	M	74	LP	LG, bilateral	Concurrent	Systemic nodes, BM, macroglobulinemia	IgΛ monotype	Not clonal	—	—	Lost to follow-up
52	F	43	ALL	Orbit	Leukemia (9)	Leukemia		Clonal	Cx	<1	DWD

F, female; M, male; DL, diffuse large B-cell lymphoma; FL, follicular lymphoma; LP, lymphoplasmacytic lymphoma; ALL, acute lymphocytoid leukemia; LG, lacrimal gland; LS, lacrimal sac; ML, malignant lymphoma; BM, bone marrow; Cx, chemotherapy; Rx, radiotherapy; DWD, died with disease; IgΛ, immunoglobulin lambda light chain.

blot analysis using a probe for the joining region of Ig heavy chain (IgH) and for the T-cell receptor (TCR) beta chain. The cases were subdivided into RLH, atypical lymphoid proliferation (borderline lesions) and lymphomas based on histomorphological and immunohistological features (Fig. 1). Definite lymphomas were classified according to the Revised European-American Lymphoma (REAL) classification (8). MALT lymphomas were diagnosed not only histologically but also by confirming the clonality by immunohistochemical Ig light-chain restriction. Immunogenotypic analysis by Southern blotting was also performed in 5 cases. Histological criteria for diagnosis of MALT lymphomas corresponded to the description by Isaacson and Norton (2). The clinical information of each patient, including therapeutic and follow-up data, was obtained from the medical records and, if needed, from physician. Primary ocular adnexal lymphoma was confined to the cases in which symptoms of the orbital lesion preceded those of the extraorbital involvement by more than 5 months and it was regarded as important that the primary orbital tumor was more larger than any other lesions at diagnosis. The anatomic localization of the lesions was defined as proposed by Knowles *et al.* (9) but was slightly modified. Briefly, ocular adnexal area was subdivided into eyelid, conjunctiva and orbit, but those involving the lacrimal glands (superolateral anterior orbit) and the lacrimal sac were documented separately.

DNA Amplification and Detection of Clonality

DNA was extracted from unstained, formalin-fixed, paraffin-embedded samples prepared according to a previously published method (10). Amplification of IgH genes was performed by seminested PCR, using primers directed to the framework 2 region and to the joining region (JH) as described previously (11). At least two DNA samples were extracted from each paraffin block and separately subjected to PCR reaction. The DNA amplification of each material was carried out more than once. The amplified products from each patient were electrophoresed in parallel. The determination as 'clonal' was made only when a single or dominant discrete band was consistently reproduced from different specimens.

Analysis of Nucleotide Sequences

DNA was recovered from the clonal bands and directly sequenced using an ABI sequencer with dye terminators (Perkin Elmer, Warington, UK). Sequence primers were identical to the primers used

TABLE 4. Reactive Lymphoid Hyperplasia

Case No.	Sex	Age (y)	Site/Bilaterality	κ/λ Light-Chain Immunophenotype	Molecular Analysis by Using PCR	Treatment	Follow-Up Time (mo)	Status at Last Follow-Up	Clinical Course
53	M	35	Orbit, bilateral	Mixed	Not clonal	Cx	103	Died of NHL	DL developed to lymph nodes and parotid glands but not to the orbit
54	M	44	Orbit	Mixed	—	Observation	31	Alive	Second biopsy showed RLH, 20 mo; Rx was conducted, 21 mo
55	M	62	LG	Mixed	Not clonal	Observation	65	Alive	
56	M	51	Orbit	Mixed	Not clonal	Observation	25	Alive	
57	F	70	LG, bilateral	Mixed	Not clonal	Observation	66	Alive	Tumor of paranasal sinus and cervical lymph node also showed RLH
58	F	55	LG, bilateral	N.D.	Not clonal	—	—	Lost to follow-up	
59	M	50	Orbit	Mixed	Not clonal	Observation	44	Alive	Second biopsy showed RLH, 42 mo
60	M	52	LG, bilateral	Mixed	Not clonal	—	—	Lost to follow-up	
61	F	47	LG, bilateral	Mixed	Not clonal	—	—	Lost to follow-up	Cervical lymphadenopathy also showed RLH
62	M	43	LG	Mixed	Not clonal	Observation	23	Alive	
63	M	28	Orbit	Mixed	—	—	—	Lost to follow-up	
64	M	31	LG	Mixed	Not clonal	Observation	18	Alive	
65	M	78	Conjunctiva	N.D.	—	—	—	Lost to follow-up	
66	F	44	LG, bilateral	Mixed	Not clonal	Cx	43	Alive	Swelling of parotid glands showed RLH
67	F	69	LG, bilateral	Mixed	Not clonal	Observation	54	Alive	
68	F	67	LG	Mixed	Not clonal	Observation	39	Alive	
69	M	69	LG	Mixed	Not clonal	Cx	36	Alive	
70	F	62	LG	Mixed	Not clonal	Observation	30	Alive	
71	M	56	Orbit	Mixed	Not clonal	Observation	22	Alive	
72	M	57	LG	Mixed	Not clonal	Observation	12	Alive	
73	F	73	LG	Mixed	Not clonal	Observation	11	Alive	
74	M	63	LG, bilateral	Mixed	Not clonal	Observation	8	Alive	

M, male; F, female; LG, lacrimal gland; N.D., not determined; Cx, chemotherapy; NHL, non-Hodgkin's lymphoma; DL, diffuse large B-cell lymphoma; Rx, radiotherapy; RLH, reactive lymphoid hyperplasia.

TABLE 5. Summary of Clinical Features of Ocular Adnexal Lymphoproliferative Disorders at Presentation

Histology	No.	M:F	Age (y)		Bilateral	Involved Sites				Extraorbital Disease	Cause-Specific Death	Initial Treatment[a]			
			Range	Mean		Conjunctiva	LG	LS	Orbit			Observation	Rx	Cx	Rx + Cx
POAL															
MALT	37	22:15	23–86	60.3	6	14	6	1	16	1	0	13	22	0	1
DL	5	3:2	45–75	65.2	1	1	2	0	2	2	1	1	1	3	0
MCL	1	1:0	69	69	1	0	0	0	1	1	1	0	0	1	0
Total	43	26:17	23–86	61.1	7	15	8	1	19	4	2	13	23	4	1
SOAL	9	4:5	43–75	60.6	2	1	2	0	6	7[b]	3	0	2	3	2
RLH	22	14:8	28–78	54.8	8	1	15	0	6	4	0	14	0	3	0

POAL, primary ocular adnexal lymphoma; DL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; MALT, MALT lymphoma; SOAL, secondary/concurrent ocular adnexal lymphoma; RLH, reactive lymphoid hyperplasia; M, male; F, female; LG, lacrimal gland; LS, lacrimal sac; Cx, chemotherapy; Rx, radiotherapy.

[a] Follow-up data were not sufficient in two, two, and five cases with MALT, SOAL, and RLH, respectively.

[b] In two of nine patients with SOALs, the lymphomatous lesions were detected only in the ocular adnexa.

in the second PCR. All sequencing was performed in both directions. The sequence data was adopted only when the results from at least two independent PCR reactions were completely consistent. The identification of the VH gene, Ig diversity (D) genes, and JH genes for germline sequences was performed by comparing those genes with the V Base, a comprehensive database of human Ig germline gene sequences compiled from published sequences (V BASE sequence directory, I.M. Tomlinson, MRC Center for Protein Engineering, Cambridge, UK). The sequence analysis software Genetyx (Software Development, Tokyo, Japan) was used. For the germline D gene attribution, the longest homology was adopted with a minimum of six successive matches or seven successive matches, interrupted by one mismatch (12).

Statistical Analysis

To compare the average ages, we used the Student's *t* test. Other comparisons were made by Fisher's exact test.

RESULTS

Clinicopathological Features

Out of 76 patients, 52 patients were diagnosed with malignant lymphomas, 22 with RLH, and 2

with atypical lymphoid proliferation since they lacked the morphological features of definite lymphoma or B-cell clonality in spite of the proliferating behavior which suggested lymphoma. All of the malignant lymphomas showed B-cell phenotype by immunohistochemistry (CD20 or CD79a) but not T-cell phenotype (CD3 or CD45RO). All of follicular lymphoma and mantle cell lymphoma displayed positivity to MAbs to Bcl-2 and Cyclin D1, respectively. The age of the patients (43 males and 33 females) ranged from 23 to 86 years (mean, 59.3 years). The follow-up period was from 0 to 120 months except for 9 patients who were lost to follow-up. The chief complaints were periorbital swelling (41%), palpable mass (28%) and irritation or pain (28%), followed by proptosis, conjunctival hyperemia, conjunctival mass and diplopia. There was no difference in terms of these complaints between those with lymphoma and those with RLH.

The frequency and clinical features of each diagnostic category are summarized in Tables 1 to 5. The most common primary lymphoma of the ocular adnexa was MALT lymphoma. The periods from the onset of symptoms to the diagnosis varied from 1 to 160 months (mean, 18.8 months) except in the case of one patient who had an extraordinarily long clinical course and had been under the observation for over 25 years as a case of 'pseudolymphoma' without disease manifestation outside the orbit (case 14). MALT lymphoma was confined to the primary site in all patients except one who presented with a clinical stage IIEA disease and who relapsed 82 months after the diagnosis (case 6). Local treatment was chosen for most patients with MALT lymphoma, but no patients died of the lymphoma during the follow-up period ranging from 1 to 120 months (mean, 32 months) except for 2 patients who were lost to follow-up. Compared to the primary non-MALT type ocular adnexal lymphoma, MALT lymphoma tended to be more localized ($P < 0.05$) and less frequently resulted in disease-related death ($P < 0.05$), but there was no statistical difference in terms of the age of the patients, the site of intraorbital involvement, bilaterality of the tumor or future relapse (Table 6).

Patients with RLH had a duration of chief complaints ranging from 2 to 132 months (mean, 18.6 months). The lacrimal gland was involved more frequently in RLH than in MALT lymphoma and other primary ocular adnexal lymphomas ($P < 0.001$). One of the patients with RLH developed systemic diffuse large B-cell lymphoma 83 months after the initial presentation but without orbital involvement (case 53). The average age of the patients with RLH, MALT lymphoma and diffuse large B-cell lymphoma was 54.8, 60.3 and 65.2 years, respectively (statistically not significant).

TABLE 6. Incidence of Clinical Parameters According to the Histological Classification of the Primary Ocular Adnexal Lymphoma

Clinical Parameters	MALT Lymphoma[a]	Lymphomas Other than MALT Lymphoma[b]	P[c]
No. of patients	36	6	
Male	21	4	NS
Site			
Bilateral involvement	6	2	NS
Confined to conjunctiva	14	1	NS
Lacrimal gland involvement	6	3	NS
Extraorbital involvement	1	2	<.05
Disease-specific death	0	2	<.05
Relapse	1	2	NS

MALT, mucosa-associated lymphoid tissue; NS, not significant.

[a] Primary ocular adnexal MALT lymphoma, excluding cases in which the lesion partially displayed a high-grade component.

[b] Includes diffuse large B-cell lymphoma and mantle cell lymphoma.

[c] Based on Fisher's exact test.

Detection of Ig Gene Rearrangement Using PCR

PCR analysis was performed on DNA extracted from the specimens of 37 cases of primary ocular malignant lymphoma and 19 of RLH. Rearranged IgH genes were detected in 57% of malignant lymphomas (55% of MALT lymphomas and 60% of diffuse large B-cell lymphomas) and 0% of RLH. In

TABLE 7. Sequence Analysis of the Immunoglobulin Heavy-Chain Genes (IgH) Expressed in the Primary Ocular Adnexal MALT Lymphoma

Case No.	Sex	Age (y)	Site/Laterality	ExtraOrbital Lesion	VH Family	Germline VH Gene	Identity[a] (%)	Germline D Gene	Germline JH Gene
6	F	55	Lacrimal sac	Hard palate	4	DP-63	81.9	D3-10	JH5
7	F	64	Conj	—	4	DP-63	96.5	D4-17	JH6
9	M	67	Orbit	—	3	DP-54	91.2	D2-2/D4/D4-b	JH5
13	M	56	Orbit	—	3	DP-54	92.5	D3-3	JH6
19	F	57	Conj (bilateral)	—	3	V3-49	94.1	D3-9	JH4
21	M	61	Orbit	—	3	DP-47	89.8	D5-18/D5-5	JH5
22	M	53	Conj	—	4	DP-63	95.1	D3-9	JH5
23	M	79	Orbit	—	3	DP-38	89.5	D2-21/D3	NI
27	M	67	Orbit	—	3	DP-53	97.2	D3-3/D3-9	JH4
28	F	44	Conj	—	3	DP-47	98.6	D2-2/D4/D4-b	JH4

Conj, conjunctiva; VH, variable gene of IgH; D, diversity gene of IgH; JH, junction gene of IgH; NI, not identified; MALT, mucosa-associated lymphoid tissue.

[a] Calculated in complementary determining region 2 and framework region 3.

TABLE 8. Frequency of VH Family Usage in Primary Ocular Adnexal MALT Lymphoma: Comparison with Low-Grade MALT Lymphoma of Other Organs and Adult Peripheral B Cells

MALT Lymphoma Arising in	No. of the Analyzed Sequences	VH1 (%)	VH2 (%)	VH3 (%)	VH4 (%)	VH5 (%)	VH6 (%)	VH7 (%)	Occurrence of Utilized DP-63 Gene within VH4 Family	
									%	(No.)
Orbital adnexa	10	0.0	0.0	70.0	30.0	0.0	0.0	0.0	100.0	(3/3)
Stomach[a]	9	0.0	0.0	55.6	44.4	0.0	0.0	0.0	50.0	(2/4)
Salivary gland[b]	29	69.0	0.0	27.6	3.4	0.0	0.0	0.0	0.0	(0/1)
Lung[c]	13	0.0	0.0	61.5	38.5	0.0	0.0	0.0	20.0	(1/5)
Adult peripheral B cells[d]		16.1	8.3	64.8	4.6	3.5	2.7	0.0		

[a] Data from Du et al. (30, 34), Chapman et al. (31), Qin et al. (28, 35), and Tierens et al. (36).
[b] Data from Bahler et al. (32, 37) and Miklos et al. (33).
[c] Data from Du et al. (30) and Kurosu et al. (29).
[d] Data from Zouali et al. (38).

a patient with diffuse large B-cell lymphoma, the right lacrimal gland morphologically exhibited an RLH pattern and immunohistologically demonstrated no Ig light chain restriction, and the left lacrimal gland displayed diffuse large B-cell lymphoma (case 38). Interestingly, however, the same monoclonal PCR band of the amplified IgH gene DNA was detected in both lesions, which implied that the RLH-like lesion of the right lacrimal gland harbored lymphoma cells (data not shown).

Analysis of VH Family Usage of MALT Lymphoma

PCR products from 10 patients with primary ocular adnexal MALT lymphoma were unselectively subjected to direct sequencing. The assignment of the sequences to VH families and germlines indicated biased usages of some germlines (Table 7). The DP-63 gene was found to be utilized in all 3 cases of MALT lymphoma using VH4 family genes. DP-54 and DP-47 genes were used in 2 of the 7 cases which expressed the VH3 families, respectively. Homology to the germline nucleotide sequences varied from 81.9% to 98.6% with an average of 92.6%. The usage of D and JH germlines seemed to be randomly distributed. The length of the CDR3 was diverse, with a mean of 23.1 nucleotides.

DISCUSSION

MALT lymphoma has been reported to occur in the ocular adnexa (13, 14). In the present study, MALT lymphoma constituted 86% of the classifiable primary ocular adnexal lymphomas. This rate is higher than the 50–64% rate in previous reports (4, 6) but is consistent with the rate reported by a study from Japan (7). This discrepancy possibly reflects the low incidence of follicular lymphoma and chronic lymphocytic lymphoma/leukemia in countries of the far east (15, 16). MALT lymphoma is a very indolent lymphoma and remains a localized disease for a long period of time. In our study, only one case (3%) showed extraorbital involvement; this incidence was lower than that of the previous reports (4, 6, 7).

Some authors have used PCR analysis to detect IgH clonality of ocular adnexal lymphoid lesions (5, 6, 14, 17–20), but it is not clear how often PCR reveals the clonality of ocular adnexal MALT lymphomas. We detected a monoclonal B-cell growth in 55% of the cases of MALT lymphoma by using PCR; in general, the PCR technique demonstrates the presence of a monoclonal B-cell population in 25 to 70% of the cases of MALT lymphoma (21).

The sequencing study of the VH gene revealed that the VH3 family was predominantly utilized in the ocular adnexal MALT lymphomas, a finding which is compatible with that of the other recent reports (22, 23). In addition, DP-63, DP-54 and DP-47, which are reported to be frequently involved in autoantibody production (24–27), were the closest germline VH genes in 7 of 10 cases examined. Frequent utilization of the germline VH genes associated with autoantibody production is also a distinctive feature of MALT lymphoma arising in other anatomical sites (28–33). These findings strongly suggest that ocular adnexal MALT lymphoma also belongs to the same category as that of marginal zone B-cell lymphoma. It is interesting to note, however, that the germline usage in the salivary gland MALT lymphoma differs quite a bit from that of our analysis (Table 8; 34–38). In the salivary gland the VH1 family genes were preferentially used and almost all of them were V1–69 (DP-10) genes (32, 33, 37) and not DP-63. In our study, all VH4 cases utilized DP-63. This distinct difference in VH gene usage does not seem to occur accidentally. Antigens which are involved in inducing chronic inflammation or autoimmune processes preceding MALT lymphoma may differ according to the anatomical sites.

In our study, one (5%) of the patients with RLH developed lymphomas afterward. Previous studies reported that 21 to 29% of patients with RLH of the ocular adnexa develop lymphomas (3, 9, 39), which is a relatively higher rate than ours. This might be due to differences in the diagnostic criteria.

The average age of patients with MALT lymphoma was 5.5 years older than that of patients with RLH. This may reflect the malignant progression of the reactive phase, although the difference in age distribution was not statistically significant ($P = 0.16$). Polito *et al.* (5) reported that some cases of orbital non-Hodgkin's lymphoma were preceded by polyclonal lymphoid hyperplasia. We found that RLH involved lacrimal glands at a significantly high rate. The lacrimal gland is often affected with chronic inflammation as is also the case in the salivary glands. The presence of autoantibodies or Sjögren's syndrome is an interesting issue to investigate, but it was not satisfactorily addressed in the present study.

Immunogenotypic studies revealed that none of the cases of RLH showed clonal B-cell proliferation. Previously, some RLH cases were reported to be clonal according to PCR (5, 17, 18). It is possible that such cases contained lymphomatous elements since some of those cases of RLH showed Dutcher's bodies or immunohistological Ig light chain restriction. Nonetheless, it should be noted that PCR may detect a small monoclonal population of lymphoid cells in the absence of clinical or histological evidence of lymphoma. Hsi *et al.* (40) reported that a monoclonal PCR pattern was present in 15% of patients with chronic active gastritis associated with *Helicobacter pylori*, although none of them developed lymphoma during a median follow-up period of 58 months, ranging from 1 to 66 months. Whether similar findings would be observed in ocular adnexal lymphoid lesions is a subject for investigation.

Low-grade MALT lymphomas sometimes show high-grade transformations. In our study, low- and high- grade components coexisted in the specimens of two cases. These cases strongly indicate that some large-cell lymphomas can originate from a high-grade transformation of MALT lymphomas, even if not all of them do. The average age of patients with diffuse large B-cell lymphoma was 4.5 years older than that of patients with MALT lymphomas, but the difference was not statistically significant.

In conclusion, the ocular adnexal lymphoproliferative disorders were diagnosed according to the REAL Classification of lymphoid neoplasms and related to the clinical data. The detection of B-cell clonality by means of PCR analysis improved the accuracy of the diagnosis. A major proportion of the primary lymphoid proliferation were divided into RLH, MALT lymphoma and diffuse large B-cell lymphoma. As reported for the gastric lymphoma, MALT lymphoma may arise from RLH and in part transform into diffuse large B-cell lymphoma, with cell proliferation presumably driven by stimulation of antigens involved in autoimmune mechanisms. The pathogen which triggers the neoplastic process in the ocular adnexa was considered to be different from that which triggers the MALT lymphoma of other organs based on molecular genetic analysis.

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