

Case Study

Local Recurrence as Immunoglobulin G4 (IgG4)-Related Disease 10 Years after Radiotherapy to Ocular Adnexal Extranodal Marginal Zone B-Cell Lymphoma of Mucosa-Associated Lymphoid Tissue

Toshihiko Matsuo,¹⁾ Kouichi Ichimura,²⁾ and Tadashi Yoshino²⁾

In 2000, a 48-year-old woman developed a left orbital mass with lacrimal gland involvement and then, in 2003, a right orbital mass with lacrimal gland involvement, both of which were diagnosed as extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). She underwent 30 Gy external beam radiation to bilateral orbital lesions. The lymphoma cells in both lesions did not share the same clonality, as shown by amplification by polymerase chain reaction of the immunoglobulin heavy chain gene. Immunoglobulin light chain analysis by immunohistochemistry and messenger RNA *in situ* hybridization showed λ chain monotype in the left orbital lesion but κ chain monotype in the right orbital lesion. She developed recurrent left orbital mass with high uptake on fluorodeoxyglucose positron emission tomography fused with computed tomography in 2010, and excisional biopsy disclosed the formation of follicles and infiltration with immunoglobulin G4 (IgG4)-positive plasma cells mainly in interfollicular areas. The immunoglobulin light chain analysis showed the λ chain and κ chain bitype. With the immunohistopathological diagnosis of IgG4-related disease, the serum IgG4 level was found to show elevation at 376 mg/dL, and the patient chose observation. This is the first reported case of development of IgG4-related disease after bilateral orbital MALT lymphoma with external beam radiotherapy. [*J Clin Exp Hematopathol* 51(2) : 125-133, 2011]

Keywords: IgG4-related disease, ocular adnexa, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), fluorodeoxyglucose (FDG) positron emission tomography fused with computed tomography (PET/CT), clonal (clonality)

INTRODUCTION

The ocular adnexa are main sites for both malignant lymphoma and inflammatory disease. Malignant lymphoma frequently involves bilateral lacrimal glands or conjunctivas at the same time or at different times.¹ Benign lymphoid hyperplasia, including orbital pseudotumor, is sometimes, but not always, a manifestation of immunoglobulin G4 (IgG4)-related diseases.²⁻⁶ Mikulicz disease, chronic sclerosing dacryoadenitis with sialadenitis, is an established entity of IgG4-related disease in the ocular adnexa.⁷

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), or simply MALT lymphoma, is the main pathological type of malignant lymphoma, arising in the ocular adnexa.¹ According to the general understanding of the interaction between cancer and inflammation,⁸ IgG4-related disease is suggested to serve as the background for its development, especially for ocular adnexal MALT lymphoma.⁹⁻¹³ In this report, we describe a patient who was proven to have IgG4-related disease many years after the initial development of bilateral lacrimal gland MALT lymphoma and external beam radiotherapy.

CASE REPORT

A 48-year-old woman experienced a 4-month history of redness in the left eye. She had a subconjunctival mass in the superotemporal quadrant of the bulbar conjunctiva (Fig. 1) and underwent partial resection, approached from the conjunctiva, in November 2000. With pathological diagnosis of MALT lymphoma (Fig. 2), she received 30 Gy external beam radiation to the left orbital lesion. In January 2003, orbital

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Departments of ¹⁾ Ophthalmology and ²⁾ Pathology, Okayama University Medical School, and Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama City, Japan

Address correspondence and reprint requests to: Toshihiko Matsuo, M.D., Department of Ophthalmology, Okayama University Medical School and Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Shikata-cho 2-5-1, Okayama City 700-8558, Japan

E-mail : matsuo@cc.okayama-u.ac.jp

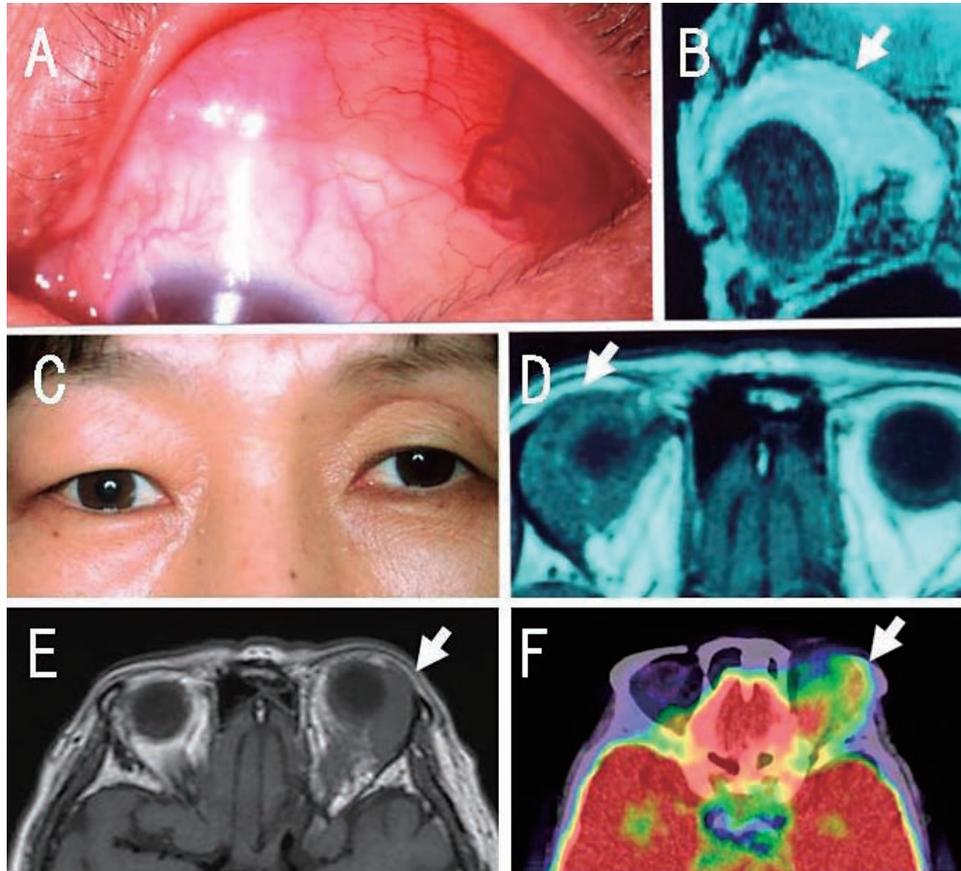


Fig. 1. Top row : An orbital lesion observed under the upper bulbar conjunctiva on the left side (1A) and magnetic resonance imaging on the sagittal plane showing a mass extending posteriorly around the eye globe to the orbital apex on the left side (arrow in 1B) in November 2000. Middle row : A mass palpable at the anterolateral edge of the orbital bone with upper lid swelling on the right side (1C) and magnetic resonance imaging on the axial plane, showing a mass extending posteriorly outside of the lacrimal gland on the right side in July 2003 (arrow in 1D). Bottom row : Magnetic resonance imaging, showing a recurrent orbital mass involving the lacrimal gland on the left side in October 2010 (arrow in 1E), which has an abnormal uptake (the maximum standardized uptake value : SUVmax = 5.35, arrow in 1F) in whole-body 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography fused with computed tomography (PET/CT).

magnetic resonance imaging showed the orbital mass involving the lacrimal gland on the right side (Fig. 1). The mass enlarged to become palpable, and she underwent partial resection with skin incision in July 2003. With pathological diagnosis of MALT lymphoma (Fig. 3), she received 30 Gy radiation to the right orbital lesion.

She was diagnosed with ulcerative colitis in 2004 and, since then, has been taking oral mesalazine at 4,000 mg daily. She underwent cataract surgery in the left eye in 2003 and in the right eye in 2006 as she developed radiation cataract. Whole-body 2-[¹⁸F] fluoro-2-deoxy-D-glucose (FDG) positron emission tomography fused with computed tomography (PET/CT), carried out as a follow-up check-up, showed no abnormal uptake in any area of the body in June 2007. In

October 2010, a mass was palpable again at the superolateral edge of the orbital bone with upper eyelid swelling on the left side, and magnetic resonance imaging revealed a recurrent orbital mass involving the lacrimal gland on the left side (Fig. 1). Repeat FDG-PET/CT demonstrated an abnormal uptake (the maximum standardized uptake value: SUVmax = 5.35) in the left orbit (Fig. 1), consistent with the recurrent mass, in the absence of other abnormal-uptake sites. She underwent excisional biopsy of the left orbital mass, approached this time by skin incision, in November 2010. IgG4-related disease was diagnosed (Fig. 4), and serum IgG4 level, measured in January 2011, was elevated to 376 mg/dL, on the basis of preliminary diagnostic criteria (higher than 135 mg/dL).¹⁴ She chose observation since the left orbital mass tended to

diminish after the second excisional biopsy. She showed no systemic manifestations, suggestive of IgG4-related disease.

METHODS

Immunohistochemistry and in situ hybridization

Pathological diagnoses were based on hematoxylin-eosin staining and immunohistochemical staining of 4- μ m-thick sections from the excised tissues fixed with 10% formalin and embedded in paraffin. Immunohistochemistry was performed and repeated for older tissues, using an automated slide stainer (BenchMark XT, Ventana Medical Systems, Inc., Tucson, Arizona, USA). Tissue sections were processed by standardized heating pretreatment for antigen retrieval prior to the usual immunohistochemical procedures.^{5,15} *In situ* hybridization was automatically performed using an integrated machine (LEICA BOND-MAX, Leica Microsystems, Newcastle Upon Tyne, UK), with fluorescein-conjugated oligonucleotide probe (Bond ISH Probe, Lambda Probe and Kappa Probe, Leica Microsystems).

The standard primary antibodies used in this study were CD20 (1:200 dilution, Novocastra, Newcastle, UK), CD3 ϵ (1:50 dilution, Novocastra), CD5 (1:100, Novocastra), CD10 (1:50 dilution, Novocastra), IgG4 (1:1,000 dilution, mouse monoclonal antibody against human IgG4, Binding Site, Birmingham, UK), and IgG (1:10,000 dilution, polyclonal rabbit antibody against human IgG, Dako, Glostrup, Denmark). Heating at 100°C for 30 min was carried out as the pretreatment for IgG4 staining while digestion with protease 1 for 4 min was performed for IgG staining. In addition, immunoglobulin light chain restrictions were examined by immunohistochemical staining for the κ chain and λ chain [mouse monoclonal antibody against human κ (1:100 dilution) and λ (1:200 dilution), Novocastra], and also by *in situ* hybridization for the κ chain and λ chain.

The number of IgG4-positive plasma cells in the areas with the highest density of IgG4-positive plasma cells was counted in a high-power field with a $\times 10$ eyepiece lens and a $\times 40$ objective lens under a light microscope. The number of IgG4-positive plasma cells in five high-power fields was counted for each specimen, and the mean proportion of IgG4-positive plasma cells among all IgG-positive plasma cells was calculated for 5 high-power fields in each specimen; a value over 40% was interpreted as positive.^{5,15}

Immunoglobulin heavy chain gene rearrangement

Immunoglobulin heavy chain gene rearrangement was detected by polymerase chain reaction (PCR),^{1,9,16} and experiments were repeated *de novo* for all three samples obtained in 2000, 2003, and 2010. Briefly, unstained, formaldehyde-fixed, paraffin sections placed on slide glasses were deparaffi-

nized with xylene and graded ethanol series, and samples for DNA isolation were cut out from at least two different areas of the deparaffinized section. The amplification of immunoglobulin heavy chain genes was performed by semi-nested PCR, using primers directed to the framework 2 region (FR2A: 5'-TGGRTCCGMCAGSCYYCNGG-3' for both the first and the second PCR) and to the joining region (LJH: 5'-TGAGGAGACGGTGACC-3' for the first PCR, and VLJH: GTGACCAGGGTNCCTTGGCCCCAG-3' for the second PCR). At least two DNA samples from each paraffin section were separately subjected to PCR with TAKARA Ex Taq (Takara Bio Inc., Otsu, Japan). The amplified products from each patient were electrophoresed in parallel in 3% agarose gel. The determination of 'clonal' was made only when a single or dominant discrete band was consistently reproduced from different specimens.^{1,16}

RESULTS

The left orbital lesion in 2000 and the right orbital lesion in 2003 shared common characteristics such as diffuse infiltration with monotonous cells that were positive for CD20, but negative for CD3, CD5, or CD10 (Fig. 2 and Fig. 3). The predominant cells were centrocyte-like cells, admixed with isolated or group-forming centroblast-like cells, and to some extent showed pseudo-follicular colonization. The left orbital tissue contained the conjunctival epithelium while the right orbital tissue contained the lacrimal gland epithelium. The monotonous cells were present beneath the conjunctival epithelium and around the lacrimal gland epithelium, respectively, but did not infiltrate inside the epithelium to form lymphoepithelial lesions in either tissue. There was no fibrosis, eosinophilic infiltration, or obliterative phlebitis in either tissue. These lines of evidence support the diagnosis of MALT lymphoma.

The analysis of immunoglobulin κ and λ light chain expression by immunohistochemistry and messenger RNA *in situ* hybridization demonstrated the light chain monotype: λ chain monotype in the left orbital lesion (Fig. 2) and κ chain monotype in the right orbital lesion (Fig. 3). IgG4-positive plasma cells were observed in the left orbital lesion and the mean proportion of IgG4-positive plasma cells among IgG-positive plasma cells was 92% (Fig. 2). In contrast, IgG4-positive plasma cells were totally absent in the right orbital lesion (Fig. 3).

In contrast with the histopathology of MALT lymphoma in the initial lesion of the left orbit (Fig. 2), the recurrent left orbital lesion in 2010 showed the formation of follicles and strand-like subdividing fibrosis, accompanied by diffuse infiltration of plasma cells mainly in interfollicular areas and also by a small number of eosinophils (Fig. 4). The orbital tissue did not contain the lacrimal gland epithelium and thus lymphoepithelial lesions were not noted. Centroblast-like cells or

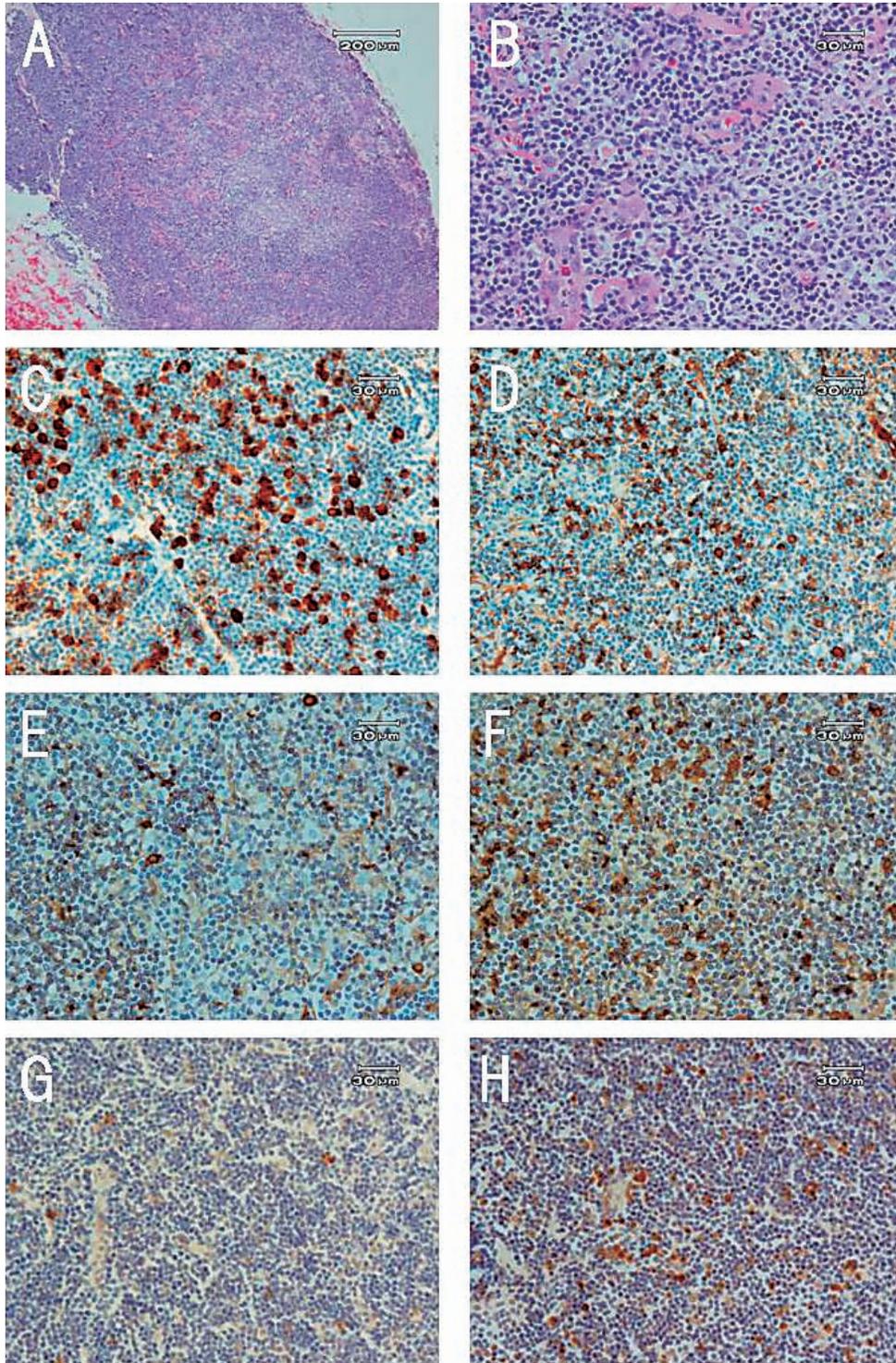


Fig. 2. Left orbital lesion in November 2000. Hematoxylin-eosin stain at lower (2A) and higher magnification (2B), showing diffuse infiltration with monotonous cells. IgG4 (2C) and IgG (2D) staining, demonstrating a high ratio of IgG4-positive plasma cells over IgG-positive plasma cells. Immunoglobulin κ chain (2E) and λ chain (2F) immunohistochemical staining as well as κ chain (2G) and λ chain (2H) messenger RNA *in situ* hybridization, revealing λ chain monotype. Bar = 30 μ m, except for bar = 200 μ m in 2A.

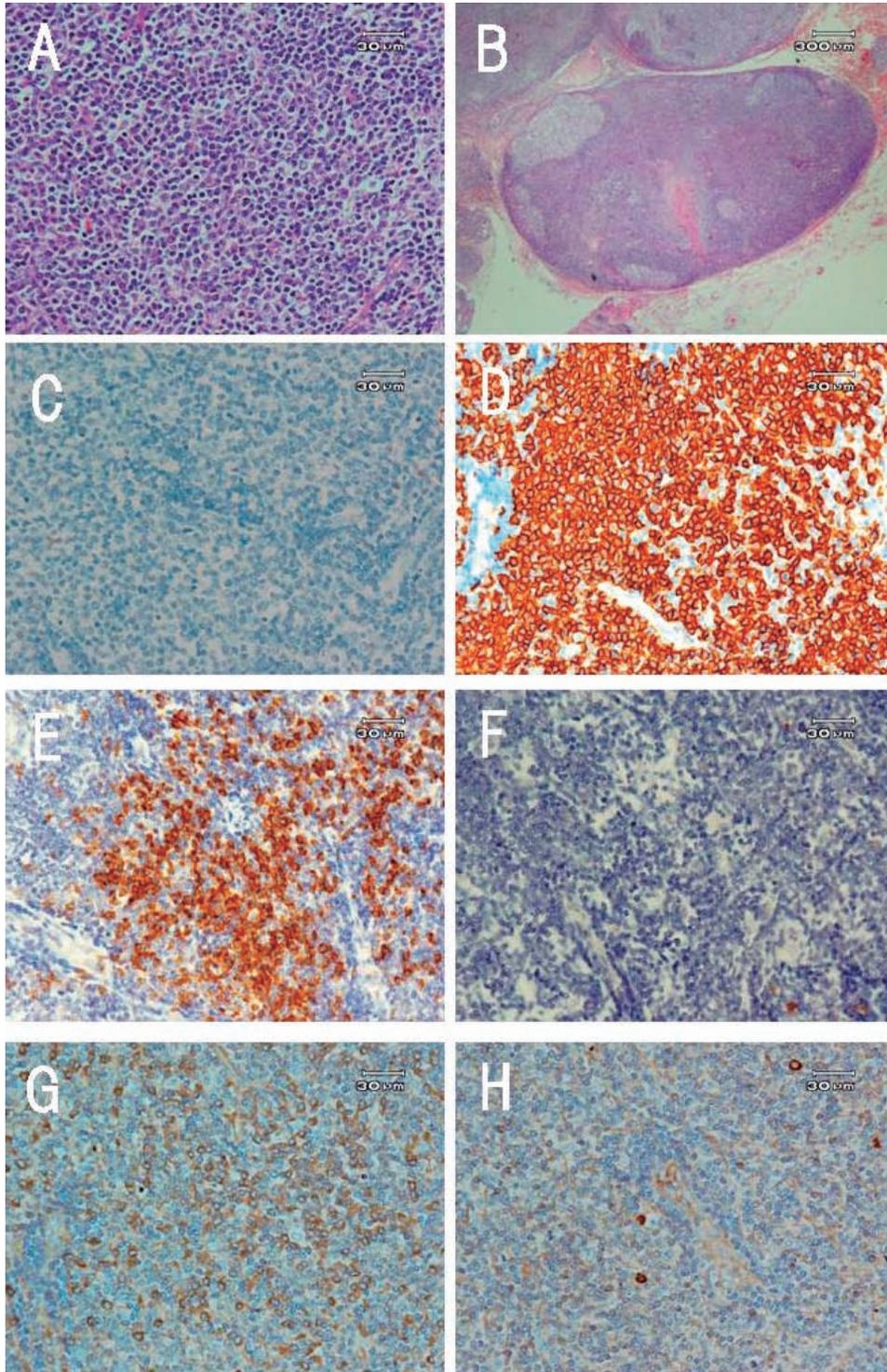


Fig. 3. Right orbital lesion in July 2003. Hematoxylin-eosin stain at higher (3A) and lower (3B) magnification, showing diffuse infiltration with monotonous cells. Cells are negative for IgG4 (3C) and positive for CD20 (3D). Immunoglobulin κ chain (3E) and λ chain (3F) immunohistochemical staining as well as κ chain (3G) and λ chain (3H) messenger RNA *in situ* hybridization show κ chain monotype. Bar = 30 μ m, except for bar = 300 μ m in 3B.

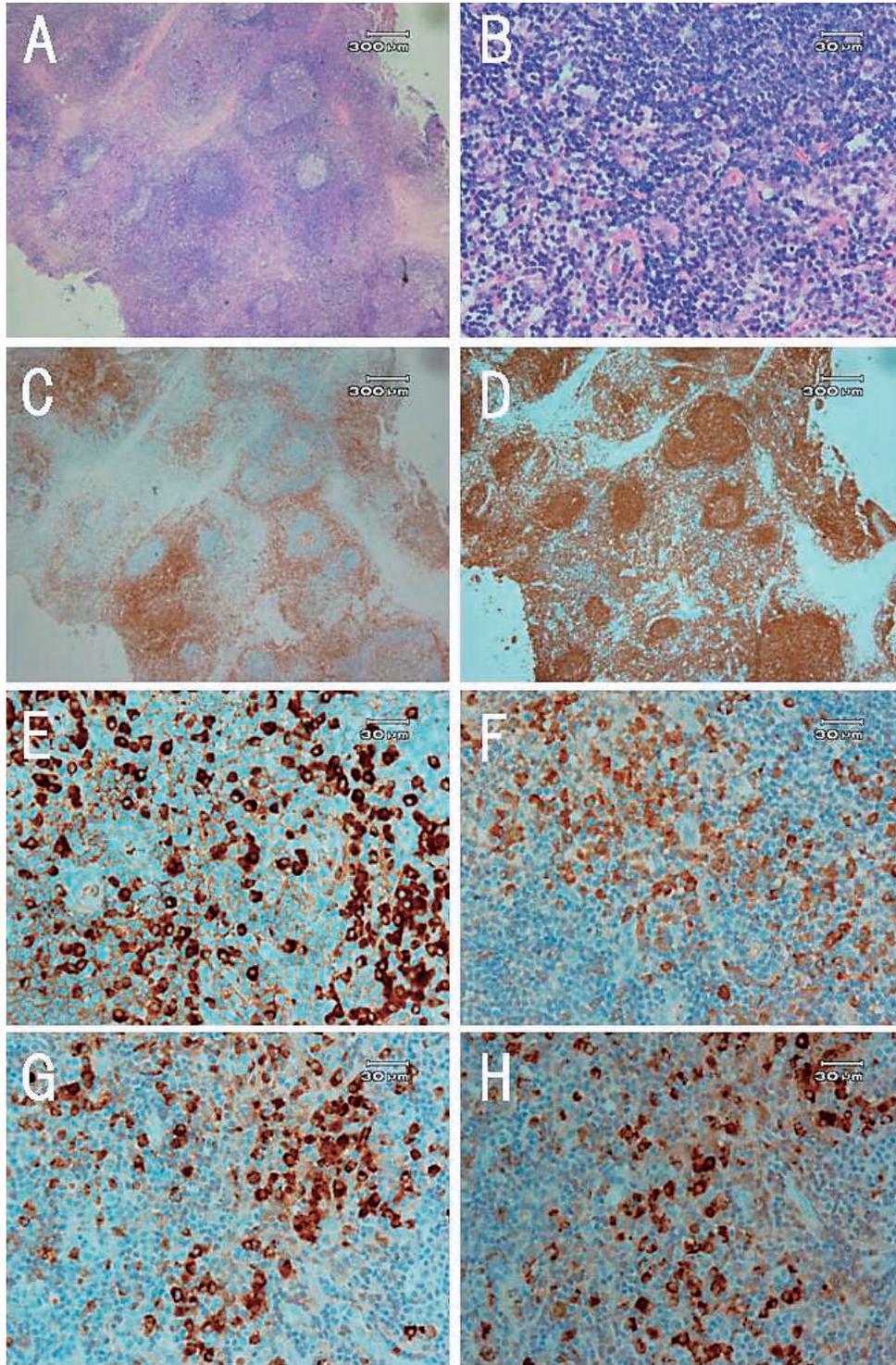


Fig. 4. Recurrent left orbital lesion in November 2010. Hematoxylin-eosin stain at lower (4A) and higher (4B) magnification, showing follicle formations and monotonous cell infiltration. CD3-positive cells (4C) infiltrate mainly in interfollicular areas while cells in the follicles are CD20-positive (4D). IgG4-positive plasma cells (4E) predominate among IgG-positive plasma cells (4F) in interfollicular areas. The cells are positive for either κ chain (4G) or λ chain (4H). Bar = 300 μ m in 4A, 4C, and 4D, while bar = 30 μ m in 4B and 4E-H.

centrocyte-like cells were absent outside the follicles. The plasma cells showed the bitype of immunoglobulin κ and λ light chain expression (Fig. 4), which refuted the clonality. The interfollicular plasma cells expressed IgG4, and the mean proportion of IgG4-positive plasma cells among all IgG-positive plasma cells was 90% (Fig. 4). A small number of IgG4-positive plasma cells were also present inside the follicles. These lines of immunohistochemical evidence led to the diagnosis of IgG4-related disease.

By PCR amplification of the immunoglobulin heavy chain gene, a single discrete DNA fragment, indicating clonality of cells, was generated from the left lesion in 2000 while one dominant fragment with another faint fragment was generated from the right orbital lesion in 2003 (Fig. 5). The single fragment on the left side and the dominant fragment on the right side had different sizes, indicating different clonalities between the left side and the right side. In contrast, an ambiguously broadened band, as a smear, was generated from two different samples of the left orbital lesion in 2010 (Fig. 5).

DISCUSSION

The goal of this study is to report the sequence of events, from the initial development of MALT lymphoma to the late development of IgG4-related disease, in the same ocular adnexa including the lacrimal gland. IgG4-related disease in the ocular adnexa has been shown to give rise to MALT lymphoma as a late sequela.¹⁰ In addition, some IgG4-producing cells were identified as neoplastic.¹¹ A similar relationship of MALT lymphoma with preceding IgG4-related disease has also been reported in the meningeal dura.¹⁷

The bilateral ocular adnexal lesions, occurring at different times in terms of initial presentation, showed that infiltrating cells had monotypic immunoglobulin light chain restriction and genomic monoclonality of the immunoglobulin heavy chain gene. In contrast, the recurrent ocular adnexal lesion on the left side showed the bitype of the immunoglobulin light chain and gave rise to a smear on PCR amplification of the immunoglobulin heavy chain gene, refuting the recurrence of MALT lymphoma.

It should be noted that the initial left orbital lesion showed λ light chain monotype while the right orbital lesion showed κ light chain monotype. *In situ* hybridization for messenger RNA of the immunoglobulin light chains in this study gave more apparent monotypic results than the immunohistochemical staining for the light chains. In addition, amplified DNA fragments of the immunoglobulin heavy chain gene generated from the bilateral orbital lesions, in a repeat experiment using new paraffin sections, had different sizes from the right side to the left side, in contrast with the previous results in which the same size was shown for both sides of the patient (designated as Case 4 in the previous report).¹

These contrasting results suggest that the clonality of neoplastic cells might be different between the right orbital lesion and the left orbital lesion, as far as the samples used in this repeat experiment are concerned. Furthermore, one discrete fragment was amplified from the immunoglobulin heavy chain gene in the previous study¹ while one dominant fragment with another faint fragment was amplified in the present study, for the same right orbital lesion of this patient. The reason for this discrepancy might be that the clonality in the right orbital lesion was different from area to area of the neoplastic tissue used for separate experiments in the previous and present studies. This study and the previous study¹ together demonstrate that amplification of the immunoglobulin heavy chain gene by PCR from a single paraffin section is a sensitive method to prove clonality of neoplastic cells, but that repeat experiments, using paraffin sections at different levels of a paraffin block, would be required to reach a more reliable conclusion.

In this patient, the ocular adnexal IgG4-related disease on the left side was preceded by MALT lymphoma with IgG4 expression. Furthermore, the IgG4-related disease developed after radiotherapy for MALT lymphoma on both sides. The cells in the right orbital lymphoma lesion showed no expression of IgG4 while the cells in the left orbital lymphoma lesion showed IgG4 expression. In other words, the right orbital lymphoma lesion did not have any IgG4-producing plasma cells while the left orbital lymphoma lesion did contain IgG4-producing plasma cells among neoplastic cells. The IgG4-producing plasma cells in the left orbital lesion might also be neoplastic since the IgG4-producing cells would be consistent with the distribution of λ light chain-restricted cells. One possibility is that IgG4-producing plasma cells served as a background for the development of MALT lymphoma and that non-neoplastic IgG4-producing plasma cells survived the radiotherapy and later grew to develop the IgG4-related disease.

The patient did not have systemic manifestations except for ulcerative colitis, which developed after the initial presentation of bilateral orbital MALT lymphoma. The serum IgG4 level, measured after the diagnosis of the orbital IgG4-related disease, showed elevation.¹⁴ She might have had subclinical IgG4-related disease for a long period of time. A so-called smoldering condition for the IgG4-related disease, if ever present, might serve as the background for the development of the orbital IgG4-related disease later in the course. Recently, inflammatory bowel disease, including ulcerative colitis, has been reported to occur in the setting of autoimmune pancreatitis, and IgG4-producing cells have been detected on one colon biopsy sample in inflammatory bowel disease.¹⁸ Ulcerative colitis in this patient might present as part of the IgG4-related disease.

FDG-PET/CT can detect both lymphoma¹ and IgG4-related disease^{5,19} in the ocular adnexa, but cannot differenti-

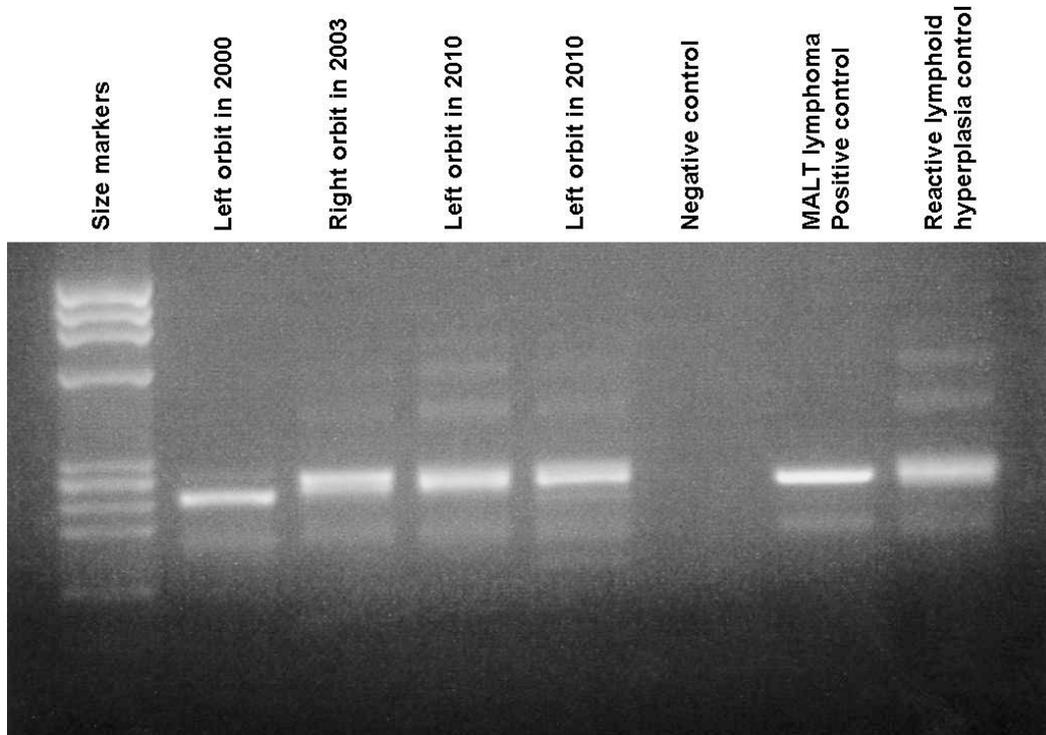


Fig. 5. Polymerase chain reaction amplification of the immunoglobulin heavy chain gene from orbital lesions. A single discrete DNA fragment, indicating clonality of cells, is generated from the left lesion in 2000 while one discrete fragment with another fainter fragment is generated from the right orbital lesion in 2003. In contrast, a main band is ambiguously broadened as a smear in two different samples from the left orbital lesion in 2010. Negative control is obtained without genomic DNA. Positive control is generated from splenic MALT lymphoma while reactive lymphoid hyperplasia control is from the pharynx. Size markers : phage X174 DNA Hae III digest (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 base pairs).

ate one from the other, as shown in the present patient. Clinical signs and imaging results, supporting the recurrence of the orbital MALT lymphoma, as shown in this patient, do not necessarily indicate lymphoma recurrence. Pathological confirmation of the lesion by biopsy is mandatory to proceed to treatment.

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