immunostaining is useful for diagnosis of this disease in addition to an elevated serum IgG4 level. Although 14 of the 23 patients did not have any pancreatic lesions, the clinical features were quite uniform and similar to those shown in AIP. Our results suggested that renal parenchymal lesions actually developed in association with IgG4-related disease, but not in association with AIP.

Tubulointerstitial nephritis is caused by various factors, including infections, drug reactions, urinary tract obstruction, autoimmune conditions, plasma cell dyscrasias, and metabolic disorders. Many patients in the present series showed hypergammaglobulinemia, hypocomplementemia, and positivity for anti-nuclear antibodies, being reminiscent of systemic lupus erythematosus, but none of them met the criteria for it. Although seven of the patients fulfilled the ordinary criteria for Sjögren's syndrome, typical Sjögren's syndrome does not show elevation of the serum IgG4 level and abundant IgG4-positive plasma cell infiltration.[3] Recent studies have shown that there are considerable differences between IgG4-related disease and Sjögren's syndrome, although distributions of the involved organs are similar,[2,3] and therefore it is important to recognize IgG4-related disease and to distinguish it from Sjögren's syndrome.[27] The clinical symptoms, laboratory findings such as hypocomplementemia, a low CRP level and negativity for ANCA, and also radiological findings, differed from those of drug-induced tubulointerstitial nephritis, infection, and ANCA-related vasculitis. The clinicopathological features of tubulointerstitial nephritis associated with IgG4-related disease are thus unique and distinct from those of other renal diseases.

Despite the characteristic clinicopathological features, the pathogenesis of IgG4-related disease remains poorly understood, although autoimmune or allergic mechanisms have been discussed.[5,19,20] Cornell *et al.*[28] demonstrated IgG4 immune-complex deposition in the renal tubule basement membranes of patients with tubulointerstitial nephritis associated with AIP, suggesting an immune-complex mechanism. In the present series, an apparent deposition of immune complex on the tubular basement membranes was evident in only one of the patients with membranous nephropathy by direct immunofluorescence. On the other hand, the relationship between glomerular lesions and IgG4-related disease is also poorly understood. Although the major pathological feature of this disease is tubulointerstitial nephritis, the glomeruli were also affected in a small number of cases in this study, and this has also been described previously,[12,15,29,30] membranous nephropathy being the most frequent feature among them. The significance of IgG4 has been documented in idiopathic membranous nephropathy, in which the predominance of Th2 cytokines is a common feature,[31,32] and also in IgG4-related disease,[5] suggesting a possible relationship between them. In view of the possible association of renal lesions with IgG4-related disease, glomerular lesions should be examined closely in addition to interstitial lesions. Further large-scale and detailed clinicopathological studies including electron microscopy examinations will be necessary to elucidate the pathogenesis of IgG4-related renal lesions.

As the concept of IgG4-related disease was proposed relatively recently, it remains largely unrecognized by most clinicians. However, accurate diagnosis of IgG4-related disease is very important because steroid therapy is usually quite effective.[19–22] In this study, the renal lesions had improved with corticosteroid therapy in most patients at the time of the 4-week follow-up. However, renal function did not recover in one patient (no. 22) with renal failure.[10] Although in this study we were unable to characterize the clinicopathological differences between patients with better or worse renal function, because it was a retrospective analysis and only one patient showed worsened renal function after treatment, it is important to be aware that renal failure may also occur in IgG4-related disease if the diagnosis is delayed. Nephrologists should be aware of the condition in patients with tubulointerstitial nephritis, and measure the serum IgG4 level, especially when there is associated sialadenitis, lymphadenopathy, hypergammaglobulinemia, eosinophilia, hypocomplementemia,[41] and a patchy lesion distribution. In addition, clinicians should be vigilant for the development of renal lesions at any time when following the course of involvement of other organs in patients with any IgG4-related disease, such as AIP. In five of the present patients, renal lesions developed during remission of the condition in other organs.

In conclusion, renal parenchymal lesions associated with IgG4-related disease appear to have characteristic clinicopathological features in comparison with those of other renal diseases, and therefore we propose the term 'IgG4-related tubulointerstitial nephritis' for this condition.

MATERIALS AND METHODS

Patients and methods

A total of 153 patients with suspected IgG4-related disease were collected retrospectively from 22 collaborating institutions in Japan between September 2004 and August 2009, among whom 30 were diagnosed as having renal parenchymal abnormalities by their physicians. Among these 30 patients, we diagnosed 23 as having renal parenchymal lesions associated with IgG4-related disease. (In Table 1, patients nos 3, 4, 5, 6, 8, 9, 13–16, 18 and 22 have been previously reported in references 10, 14, and 33–40.) The diagnosis was based on a high serum IgG4 level (>135 mg/dl) and numerous infiltration of IgG4-positive plasma cells into the renal interstitium (IgG4-positive plasma cells/IgG-positive plasma cells $>40\%$; IgG4-positive plasma cells >10 per h.p.f.) with fibrosis. Although the serum IgG4 level was not measured in one patient, we also diagnosed this patient as having renal parenchymal involvement associated with IgG4-related disease because of the presence of tubulointerstitial nephritis with infiltration of numerous IgG4-positive plasma cells into the renal interstitium with typical AIP and Mikulicz's disease. In the other 7 of 30 patients, renal parenchymal abnormalities were diagnosed on the basis of radiographic abnormalities. Five of these patients were diagnosed as having IgG4-related sialadenitis and dacryoadenitis and showed multiple hypoattenuating lesions in the renal cortex by contrast-enhanced

CT, with normal urinalysis values and normal renal function. One other patient (under hemodialysis) was diagnosed as having IgG4-related sclerosing cholangitis and end-stage renal disease, with evidence of a mass lesion in the right kidney by nonenhanced CT. Renal biopsy was not performed in these six patients. Another patient showed a right renal mass lesion and a high serum IgG4 level, with normal urinalysis values and mild renal dysfunction. As the tissue obtained by CT-guided renal biopsy of the mass lesion did not include any renal parenchyma, even though marked lymphoplasmacytic infiltration was observed in the adipose tissues, we excluded this patient.

In the 23 patients diagnosed as having renal parenchymal lesions associated with IgG4-related disease, we retrospectively examined the clinical features, data from laboratory and imaging studies, and the clinical response to treatment. Whole-body CT imaging was evaluated in all patients (contrast-enhanced CT in 21 patients). Gallium citrate scintigraphy was performed in 19 of the 23 patients, before therapy in 17 and during therapy in 2. The diagnosis of AIP was made in accordance with the 2006 Japan Pancreas Society revised criteria.[42] Sialadenitis and dacryoadenitis were diagnosed on the basis of physical findings, and the results of imaging studies (CT and gallium citrate scintigraphy) and/or biopsy.

The study was approved by the review board of the Nagaoka Red Cross Hospital and the boards of the various collaborating institutions. All data and samples from patients were collected with their informed consent, and the research was conducted in compliance with the principles of the Declaration of Helsinki.

Renal pathology

Renal pathological examination was conducted at the request of the attending physicians because of urinary abnormalities, renal dysfunction, and/or radiological abnormalities. Renal tissues were obtained by nondirected medical needle biopsy in 21 patients, by open biopsy of the mass lesion in 1 patient (no. 8), and by autopsy in 1 patient (no. 3), who died of lung cancer. All renal tissue specimens were examined by light microscopy. Direct immunofluorescence studies were conducted in 17 patients, and we evaluated 14 specimens because 3 were considered inadequate. For routine light microscopy studies, renal biopsy specimens were fixed in formalin or alcohol-Bouin, embedded in paraffin, and stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-methenamine silver, and Masson's trichrome. For immunostaining, formalin-fixed, paraffin-embedded biopsy specimens were cut into 3-μm thick sections, and the sections were immunostained using anti-IgG antibody (Dako, Glostrup, Denmark) and mouse monoclonal antibody against human IgG4 (Zymed Laboratory, San Francisco, CA, USA, or The Binding Site, Birmingham, UK). For direct immunofluorescence studies, tissues were snap frozen, and sections were treated with fluorescein isothiocyanate-conjugated rabbit anti-human IgG, IgA, IgM, C1q, C3c, or fibrinogen (Dako, Carpinteria, CA, USA).

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

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Original Article

Atypical Lymphoplasmacytic and Immunoblastic Proliferation of Autoimmune Disease : Clinicopathologic and Immunohistochemical Study of 9 Cases

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Atypical lymphoplasmacytic immunoblastic proliferation (ALPIB) is a rare lymphoproliferative disorder (LPD) associated with autoimmune disease (AID). To further clarify the clinicopathologic, immunohistological, and genotypic findings of ALPIB in lymph nodes associated with well-documented AIDs, 9 cases are presented. These 9 patients consisted of 4 patients with systemic lupus erythematosus, 3 patients with rheumatoid arthritis, and one case each with Sjögren's syndrome and dermatomyositis. All 9 patients were females aged from 25 to 71 years with a median age of 49 years. Four cases presented with lymphadenopathy as the initial manifestation. In 4 patients, immunosuppressive drugs were administered before the onset of lymph node lesion. However, none of the 9 patients received methotrexate therapy. The present 9 cases were characterized by : (i) prominent lymphoplasmacytic and B-immunoblastic infiltration ; (ii) absence of pronounced arborizing vascular proliferation ; (iii) absence of $CD10^+$ "clear cells" ; (iv) presence of hyperplastic germinal center in 7 cases ; (v) immunohistochemistry, flow cytometry, and polymerase chain reaction demonstrated a reactive nature of the T- and B-lymphocytes ; and (vi) on *in situ* hybridization, there were no Epstein-Barr virus -infected lymphoid cells in any of the 9 cases. Overall 5-year survival of our patients was 83%. The combination of clinical, immunophenotypic, and genotypic findings indicated that the present 9 cases can be regarded as having an essentially benign reactive process. Finally, we emphasized that ALPIB should be added to the differential diagnostic problems of atypical LPDs, particularly lymph node lesions of IgG4-related diseases. [*J Clin Exp Hematopathol 50(2) : 113-119, 2010*]

Keywords: autoimmune disease, lymphadenopathy/atypical lymphoplasmacytic and immunoblastic proliferation, IgG4-related disease

INTRODUCTION

Reactive lymph node lesions in patients with autoimmune disease (AID) and its related disorders exhibit marked histological diversity and are occasionally associated with atypical lymphoproliferative disorders (LPDs).[1,2] In 1984, Koo *et al.* reported an unusual lymph node lesion associated with various AIDs including systemic lupus erythematosus, rheumatoid arthritis (RA), Sjögren's syndrome, and autoimmune hemolytic anemia.[3] Histopathologically, the lesion was characterized by prominent lymphoplasmacytic infiltration with various numbers of immunoblasts, namely, atypical lymphoplasmacytic and immunoblastic proliferation (ALPIB).[3] Although ALPIB is a rare lymphoproliferative disorder associated with AIDs, it occasionally presents serious problems in the differential diagnosis from atypical or malignant LPDs containing numerous plasma cells and immunoblasts, and

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exhibiting autoimmune disease-like clinical manifestations, particularly angioimmunoblastic T-cell lymphoma (AITL). However, except for an earlier report by Koo *et al.*,[3] only sporadic case reports have been published.[4-7] Previously, we reported clinicopathologic findings of 5 such cases.[5-7] Recently, several authors have emphasized the differential diagnostic problems for ALPIB from lymph node lesions of IgG4-related diseases.[8-10] Because limited clinicopathological information is available for ALPIB, the present study documented essential data in 9 patients with ALPIB due to AIDs and discussed the differential diagnostic problems between ALPIB and atypical LPDs including lymph node lesions of IgG4-related disorders.

MATERIAL AND METHODS

Nine cases were collected from a series treated by one of the authors (M.K.) between 1999 and 2009. Medical records of these 9 cases were extensively reviewed. Five cases (nos. 2, 4, 5, 8, and 9) were reported previously.[5-7]

Tissue specimens were fixed in formalin solution, routinely processed, and embedded in paraffin. For light microscopic examination, the sections were stained with hematoxylin-eosin.

Immunohistochemical studies were performed using Ventana automated stainer (BenchMark™, Tucson, Arizona, USA) or Histofine Histostainer (Nichirei Bioscience Inc., Tokyo, Japan) according to the manufacturer's instructions.

A panel of antibodies against human immunoglobulin light chains (κ and λ) (Novocastra, Newcastle, UK, or Nichirei Co., Tokyo, Japan), IgG (Novocastra), IgA (Novocastra), IgM (Novocastra), IgG4 (MCC011 ; Binding Site, Birmingham, UK), CD3 (PS-1 ; Immunotech, Marseille, France), CD5 (4C7 ; Novocastra), CD15 (C3D-1 ; Dako A/S, Glostrup, Denmark), CD20 (L26 ; Dako A/S), cocktail of CD21 (2G9 ; Novocastra), CD35 (RB L25 ; Novocastra), CD30 (Ber-H2 ; Dako A/S), CD43 (DFT-1 ; Dako), antifollicular dendritic cell antibody CNA 42 ; Dako), and human-herpes virus-8 (137B1 ; Novocastra) were used. Sections with known reactivity for the antibodies assayed served as positive controls and sections treated with normal rabbit and mouse serum served as negative controls.

In situ hybridization (ISH) with Epstein-Barr virus (EBV)-encoded small RNA (EBER) oligonucleotides was performed to test for the presence of EBV small RNA in formalin-fixed paraffin-embedded sections using a Ventana automated (BenchMark™) stainer or a hybridization kit (Dako).

Genomic DNA was extracted from formalin-fixed tissues after dewaxing of paraffin sections, then immunoglobulin heavy chain (8 cases : nos. 1-8) and T-cell receptor (TCR) γ-chain gene (4 cases : nos. 2, 4, 5, and 8) rearrangements were analyzed by polymerase chain reaction (PCR) as described previously.[11,12]

Actuarial overall survival curve distributions were calculated by the Kaplan Meier method.[13]

RESULTS

The main clinicopathologic findings are shown in Tables 1 and 2.

Clinical findings

All 9 patients were females and ranged in age from 25 to 71 years, with a mean age of 49 and a median age of 49. At the time of the initial lymph node biopsy, 4 cases (nos. 1-3 and 7) fulfilled the diagnostic criteria for systemic lupus erythematosus,[14] while 3 cases (4, 8, and 9) were diagnosed as RA,[15] and 1 each was diagnosed as dermatomyositis (no. 5)[16] and Sjögren's syndrome (no. 6).[17]

AIDs were active in 7 patients (nos. 1, 4-9) and inactive in only 2 patients (nos. 2 and 3). Seven patients (nos. 1, 2, 4-7, and 9) had constitutional symptoms such as fever at lymph node biopsy. Four cases (nos. 1 and 5-7) presented with lymphadenopathy at the onset of disease. Multicentric lymph node enlargement was present in 6 cases (nos. 4-9). Analysis of patient lifestyles did not suggest any risk factors for human immunodeficiency virus type-1 infection, although serological data on anti-human immunodeficiency virus type-1 antibody were available in only 4 cases (nos. 1, 3, 6, and 7). Polyclonal hyper-γ-globulinemia was observed in 5 cases (nos. 3, 5-7, and 9). Various autoantibodies including positive rheumatoid factor and antinuclear antibody were detected in all but one case (no. 5). The level of total functional hemolytic complement (CH50) was decreased in 2 (nos. 1 and 7) of the 7 cases (nos. 1-4, 7-9) examined. Serum IgG4-level was examined in 3 cases (nos. 3, 6, and 7). Serum IgG4 level was within the normal range (< 135 mg/dL) in 2 cases (nos. 3 and 6), whereas elevated serum IgG4 level (221 mg/dL) was recorded in the remaining 1 case (no. 7). However, elevated interleukin-6 (IL-6) level was also recorded in Case 7 (19 pg/mL, normal range < 4.62).

All 5 cases (nos. 2-4, 6, and 7) with analyzable metaphases had a normal karyotype. Information from flow cytometry of the biopsied specimens showed a polyclonal B-cell population in 6 cases (nos. 2-4, 6, 7, and 9) examined. There was no absence of pan-T-cell markers in any of the 6 cases examined.

At lymph node biopsy, 3 patients (nos. 2-4) were receiving steroid therapy, and 1 patient each was receiving nonsteroidal anti-inflammatory drug (no. 1), gold therapy (no. 8), and mizoribine (no. 9). However, none of the 9 patients received methotrexate (MTX) therapy. The remaining 3 cases (no. 5-7) had not received any medications, but soon after the lymph node biopsy, all 3 of these patients were treated with predni-

Table 1. Summary of clinical findings

No	Age/ gender	Disease	Disease activity	Site of lymph node swelling	Interval (months)	Symptom and sign	Abnormal laboratory findings	Immunosup-pressive therapy	Outcome
1	25/F	SLE	Active	Bilateral neck	Onset	Fever, skin rash, convulsion, arthralgia	Leukopenia, ANA (+), hypocomplementemia	—	CHOP, 6 m, recurrence, 34 m, died with sepsis
2	30/F	SLE	Inactive	Right neck	216	Fever	Anemia, leukocytosis, ADNA (+)	β-methasone	β-methasone, 69 m, alive
3	39/F	SLE	Inactive	Bilateral neck	240	—	Anemia, ANA (+), SS-A/ b antibody (+), polyclonal hyper-γ-globulinemia	Prednisolone	Prednisolone 1 m, alive
4	48/F	RA	Active	Systemic	120	Fever	RF (+)	Prednisolone	Prednisolone 48 m, Alive
5	49/F	DM	Active	Left axilla & groin	Onset	Fever, general fatigue	↑ creatine kinase, ↑ aldolase, polyclonal hyper-γ-globulinemia	—	Prednisolone 120 m, alive
6	50/F	SJS	Active	Systemic	Onset	Fever, dry eye & mouth	ANA (+), polyclonal hyper-γ-globulinemia	—	Prednisolone, 20 m, alive
7	61/F	SLE	Active	Systemic	Onset	Body weight loss	ANA (+), polyclonal hyper-γ-globulinemia, hypocomplementemia	—	25 m, alive
8	68/F	RA	Active	Right neck & mediastinum	72	—	RF (+), ANA (+)	—	Gold therapy 57 m, alive
9	71/F	RA	Active	Left neck & bilateral axilla	276	Fever, arthralgia, pulmpnary infiltration	RF, ANA (+), polyclonal hyper-γ-globulinemia,	Mizoribine	Mizoribine+, prednisolone, lost

Abbreviations: Interval, interval between onset of disease and lymphadenopathy; SLE, systemic lupus erythematosus; DM, dermatomyositis; RA, rheumatoid arthritis; SJS, Sjögren's syndrome; ANA, anti-nuclear antibody; ADNA, anti-DNA antibody; RF, rheumatoid factor; CHOP, cyclophosphamide, doxorubicin, vincristine, & prednisone; m, months

Table 2. Summary of pathological findings

	Size (cm)	Follicles		Interfollicular area							Sinus
		Folicular hyperplasia	Follicular dendritic cell network	Small vessels	Plasma cells	Immunoblasts	Monocytoid B-cells	Neutrophils	Eosinophils	Histiocytes	
1	1.0	—	—	+	+	+++	—	+	+	++	obliterated
2	1.5	—	abnormal proliferation	++	++	++	—	—	+	+	obliterated
3	1.8	++	normal	+	++	++	+	—	+	+	apparent
4	2.5	++	normal	++	++	+++	+	—	—	+	apparent
5	1.2	+	broken up	+	+++	+	—	—	—	+	apparent
6	3.0	++	broken up	++	+++	+	—	—	—	+	apparent
7	1.5	++	normal	+	+++	+	—	—	—	+	apparent
8	1.2	++	broken up	++	+	+	—	—	+	++	obliterated
9	1.0	++	broken up	++	++	++	—	++	—	++	apparent

—, negative, only a few or absent; +, scattered or mild; ++, moderate; +++, numerous or prominent

solone. One patient (no. 8) was treated with mizoribine and prednisolone. Four patients (nos. 2-4 and 9) continued to receive the same therapeutic agents as before the lymph node biopsy. The remaining one case (no. 1) received CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) therapy, because clinically malignant lymphoma was highly suspected.

Follow-up data were obtained from 8 cases (nos. 1-8). None of the 8 surviving cases developed malignant lymphoma during the follow-up period from 1 to 120 months (mean, 47 mon ; median, 41 mon). One patient (no. 1) developed recurrent lymph node swelling after 6 months and died with sepsis after 34 months, while the remaining 7 cases were alive at the last follow-up. The overall survival rate of the 8 cases was 83% at 5 years (Fig. 1).

Pathological, immunohistochemical, and EBV findings

All enlarged lymph nodes had a diameter of less than 3.0 cm. On low-power field, the biopsy specimens were characterized by obvious paracortical expansion with diffuse effaced lymph node architecture (Fig. 2a). Lymphoid follicles were seen in 7 cases (nos. 3-9), and their germinal centers were usually hyperplastic, although a few were rather atrophic.

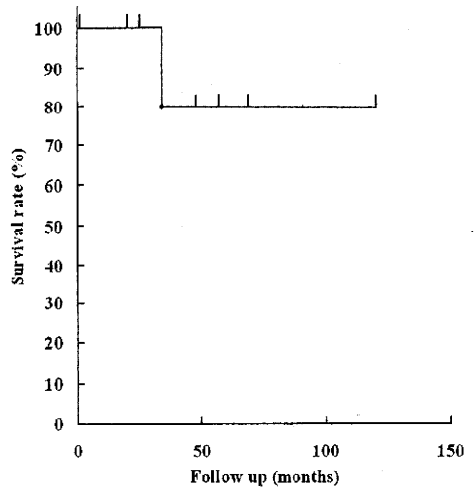

Fig. 1. Overall survival of the 8 patients with atypical lymphoplasmacytic immunoblastic proliferation.

Each case contained mild to moderate small vessels in the interfollicular area. Lymphoid sinuses appeared to be obliterated in 3 cases (nos. 1, 2, and 8). Perivascular fibrous masses were observed in 1 case (no. 9).

On high-power field, the paracortical area was diffusely infiltrated by a polymorphous population consisting of numerous mature plasma cells, plasmacytoid cells, large basophilic transformed lymphocytes (immunoblasts), and small- to medium-sized lymphocytes (Fig. 2b). A proportion of immunoblasts with large vesicular nuclei and prominent nucleoli resembling Hodgkin cells were observed in 4 cases (nos. 2, 4, 8, and 9), but typical Reed-Sternberg cells were not detected (Fig. 2b). Small- to medium-sized lymphocytes exhibited minimal cytological atypia. There were no medium to large lymphoid cells with clear cytoplasm (clear cells). Moderate numbers of histiocytes with or without epithelioid cell features were seen in 3 cases (nos. 1, 8, and 9).

Scattered eosinophils were observed in 4 cases (nos. 1-3 and 8) (Fig. 2b). In the paracortical area, small vessels usually had plump nuclei, however, high endothelial venules showing arborization were not prominent. In 2 cases (nos. 3 and 4), foci of monocytoid B-cells were seen.

Staining with CD20, CD3, and CD5 showed a mixture of small- and medium-sized lymphocytes. The majority of immunoblasts in 5 cases (nos. 1, 4, 5, 8, and 9) showed the B-cell phenotype (Fig. 2c, d). The other 4 cases (nos. 2, 3, 6, and 7) showed polytypic immunoblasts. A proportion of the B-immunoblasts were CD30-positive but CD15-negative. There were no CD43-positive lymphocytes detected. In the interfollicular area, 60% of these paracortical T cells expressed CD4, and the remaining 40% were CD8-positive in the 3 cases examined (nos. 1, 2, and 8). Immunohistochemical studies of light chain determinants for interfollicular plasma cells, plasmacytoid cells, and B-immunoblasts demonstrated a polyclonal pattern (Fig. 2e, f).

A monoclonal antibody cocktail of 2G9 and RB L25, as well as CNA 42 highlighted the meshwork of follicular dendritic cells (FDCs). The FDC meshwork maintained a regular arrangement in 3 cases (nos. 3, 4, and 7), whereas a few of the meshworks were broken up into clusters in 4 cases (nos. 5, 6, 8, and 9) (Fig. 2g). In 1 case (no. 2), a monoclonal antibody cocktail of 2G9 and RB L25 demonstrated scattered large irregularly shaped accumulations of FDCs surrounding the small vessels (Fig. 2h). The remaining 1 lesion (no. 1) did not contain FDC meshwork.

There were no human-herpes virus-8- or EBER-positive cells in any of the 9 cases.

Immunogenotypic results

PCR assays for *TCR-γ* and/or *IgH* genes were performed in 8 cases (nos. 1-8). None of 4 cases (nos. 2, 4, 5, and 8) demonstrated clonal bands on TCR-γ PCR. PCR assay for *IgH* gene demonstrated only germline bands with IgH chain probes in 8 cases (nos. 1-8).

DISCUSSION

The clinical manifestations of our 9 cases including multicentric lymphadenopathy with systemic symptoms and abnormal immunological findings raised the possibility of malignant lymphoma, prompting a lymph node biopsy. Histologically, the present 9 cases were characterized by : (i) prominent lymphoplasmacytic and B-immunoblastic infiltration ; (ii) an absence of pronounced arborizing vascular proliferation ; (iii) absence of $CD10^+$ "clear cells" ; (iv) presence of hyperplastic germinal center in 7 cases ; (v) a polyclonal nature of both T- and B-lymphocytes on immunophenotypic and genotypic analysis ; (vi) flowcytometry demonstrated that there was no absence of pan T-cell marker, which is a characteristic flowcytometric finding of AITL ;[18] and (vii) EBV-infected lymphoid cells were absent in all 9 cases. The overall histologic, immunohistochemical, genotypic, and EBV findings of this case were similar to those of previous reports,[3-7] and the present 9 cases may be classified as ALPIB associated with AIDs.

The present 9 cases indicate that ALPIB should be differentiated from various atypical and malignant LPDs containing numerous B-immunoblasts, plasma cells, and cells with plasma cell differentiation and exhibiting AID-like clinical manifestations.

The differential diagnostic problems between ALPIB and non-Hodgkin's, and in particular AILT, have been well described in the literature.[5-7,9,10,19,20] AITL rarely occurs in systemic rheumatic diseases such as RA.[21] However, histological, immunohistochemical, flowcytometric, and genotypic

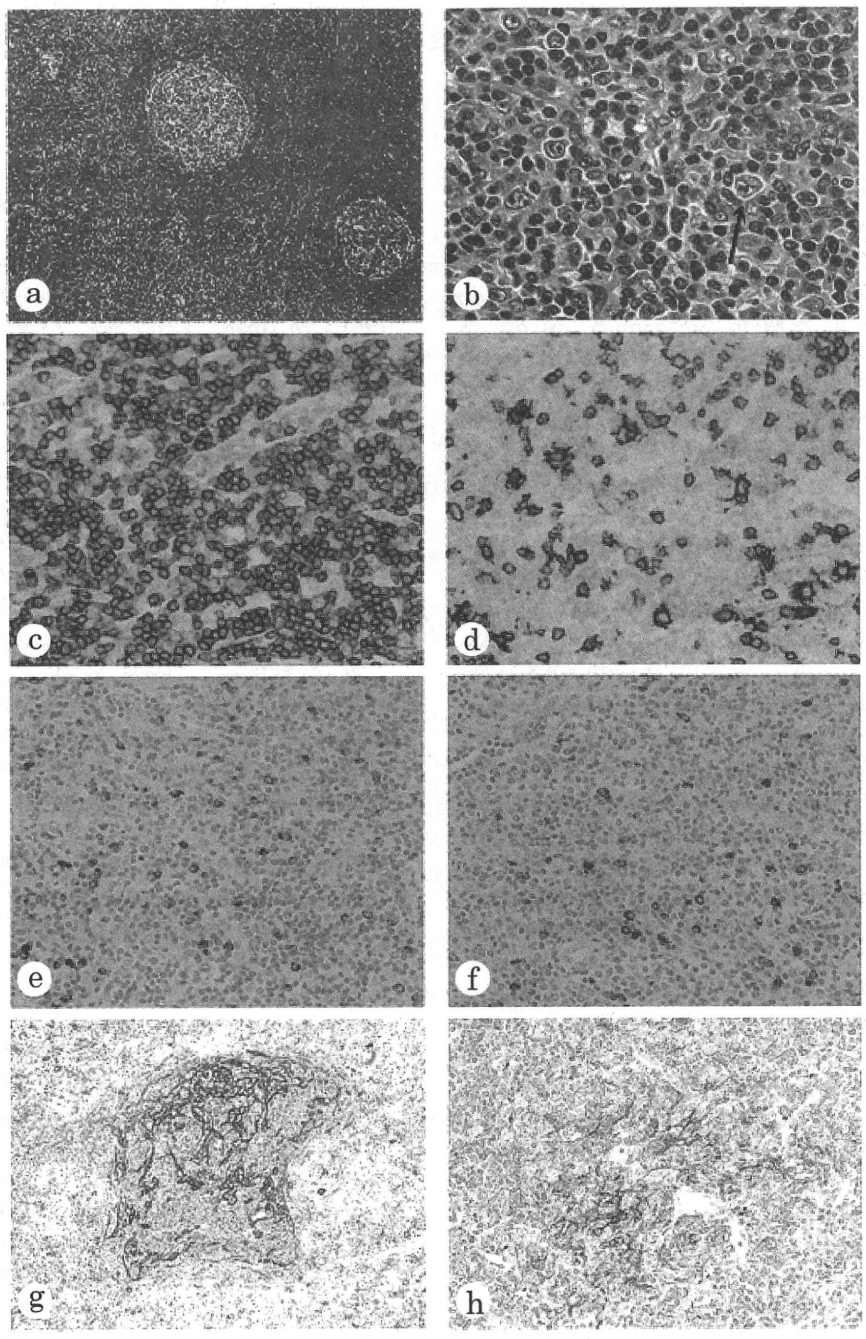

Fig. 2. Histological and immunohistochemical findings of atypical lymphoplasmacytic immunoblastic proliferation. (***2a***) Low-power field of biopsy specimen. The lesion was characterized by diffuse paracortical hyperplasia with small vessel proliferation and a hyperplastic germinal center. Case 7, H&E stain, ×25. (***2b***) On high-power field, the paracortical area contained mature plasma cells, plasmacytoid cells, immunoblasts, small- and medium-sized lymphocytes, and an eosinophil. Note a Hodgkin-like cell (*arrow*). Case 2, H&E stain, ×150. (***2c***) & (***2d***) Immunohistochemical study demonstrated that the majority of small- and medium-sized lymphocytes were positive for CD5 (*2c*), whereas immunoblasts were usually positive for CD20 (*2d*). Case 4, counterstained with hematoxylin, ×100. (***2e***) & (*2f*) Immunostaining for light chain determinant of the immunoglobulins demonstrated the polytypic nature of the plasma cells and their precursors κ (*2e*) and λ (*2f*). Case 2, counterstained with hematoxylin, ×100. (***2g***) Staining with CNA 42 highlighted the broken follicular dendritic cell meshwork. Case 8, counterstained with hematoxylin, ×25. (***2h***) Staining with a monoclonal antibody cocktail of 2G9 and RBL25 highlighted the large irregularly shaped accumulations of follicular dendritic cells surrounding the small vessels. Case 2, counterstained with hematoxylin, ×50.

study demonstrated the non-neoplastic nature of the present 9 cases.

Little attention has been paid to the differential diagnostic problem between ALPIB and atypical LPDs.[5-7] Among the few reported studies in the Far East including Japan, lymph node lesions in IgG4-related disease appear to be the most important differential diagnostic problem.[8-10] Histologically, a proportion of the lymph node lesions in IgG4-related disease are characterized by prominent lymphoplasmacytic infiltration with various numbers of immunoblasts.[9,10] However, there was no evidence of definite autoimmune disease in IgG4-related disease.[8] Interestingly, elevated serum IgG4 level was detected in 1 (no. 7) of 3 cases examined. However, elevated serum IL-6 level was also recorded in Case 7. Autoimmune disease is a form of IL-6 disorder.[22] It is well known that human IgG4 production is regulated by IL-6.[23]

In middle-aged and elderly patients, EBV-associated reactive lymph node lesions rarely exhibit autoimmune disease-like clinical manifestations.[24] Histologically, lymph node lesions in these patients were similar to those of ALPIB in some aspects. The biopsy specimens contained numerous lymphoid follicles with hyperplastic germinal centers and pronounced arborizing vasculature in the expanded paracortex. The paracortical area contained polymorphic infiltrates with numerous small- and medium-sized lymphocytes and plasma cells, and variable numbers of immunoblasts, epithelioid histiocytes, and occasional eosinophils. However, their autoimmune disease-like clinical manifestations were usually transient. Moreover, there were no EBER$^+$ cells in any of the present 9 cases.

Since the early 1990s, atypical or malignant LPDs in patients immunosuppressed with MTX for treatment of RA have been reported.[25-28] A proportion of MTX-induced LPD exhibited atypical lymphoplasmacytic infiltrations showing histological findings similar to those in our cases including the presence of (i) an expanded paracortical area consisting of a mixed cell population, including small- and medium-sized lymphocytes, plasmacytoid lymphocytes, and immunoblasts, and (ii) immunoblasts including Hodgkin-like cells usually showing a B-cell phenotype, and a proportion of these cells were CD30-positive but CD15-negative.[27,28] However, there was no history of MTX therapy in the present 9 cases.

As previously suggested by Blanco *et al.*,[4] the absence of EBV as determined by ISH studies in all our 9 cases indicates that EBV was not related to the lymphoproliferative process in the majority of ALPIB cases. Moreover, the present study suggests that the underlying cause of lymphadenopathy in these ALPIB patients may involve the chronic immune problems caused by AID.

Overall 5-year survival of our series was 83%. The combination of clinical, immunophenotypic, and genotypic findings indicated that the present cases should be regarded as having an essentially benign reactive process. However, 4 of our 9 cases demonstrated lymphadenopathy at the onset of autoimmune disease. We emphasize that ALPIB should be differentiated from various LPDS showing autoimmune disease-like clinical findings.

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Epstein-Barr Virus-Related Atypical Lymphoproliferative Disorders in Waldeyer's Ring: A Clinicopathological Study of 9 Cases

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Key Words
Epstein-Barr virus · Waldeyer's ring · Lymphoproliferative disorders

Abstract
Background and Study Aim: Because of the small biopsy specimens in the Waldeyer's ring (WR) the differential diagnosis between Epstein-Barr virus (EBV)-associated lymphoproliferative disorder (LPD) and malignant lymphoma is occasionally difficult. We report here clinicopathological, immunohistochemical and genotypic findings of 9 cases of EBV-associated LPDs in WR. ***Patients and Methods:*** Using formalin-fixed paraffin-embedded sections, histological analyses, immunohistochemistry, in situ hybridization and polymerase chain reaction were performed. ***Results:*** Clinically, all 9 cases showed more than one atypical clinical finding of infectious mononucleosis including absence of systemic symptoms, absence of atypical lymphocytosis and age over 30 years. Histologically, 3 types were delineated: (1) Hodgkin lymphoma-like (n = 1), (2) T cell/histiocyte-rich large B cell lymphoma-like (n = 4), and (3) marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT)-like (n = 4). The in situ hybridization study demonstrated EBV-encoded small RNA (EBER)+ cells in all 9 lesions. The immunohistochemical and flow cytometry study demonstrated the reactive nature of the B cells in all 9 lesions. However, 3 of our 7 cases examined demonstrated immunoglobulin heavy chain gene rearrangement on PCR study. There was no development of B cell lymphoma in any of the 3 lesions demonstrating IgH rearrangement. ***Conclusion:*** EBV-associated LPDs of the WR showed marked histological diversity. Among these, a MALT-like pattern was frequently seen. Marginal zone B cell lymphoma frequently affects WR. We emphasized that EBV-associated LPD should be added to the differential diagnosis of primary tonsillar MALT-type lymphoma.

Introduction

Infectious mononucleosis (IM) is an acute lymphoproliferative disorder (LPD) that typically occurs in young patients and is usually caused by Epstein-Barr virus (EBV) [1, 2]. The diagnosis of IM is usually based on

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Table 1. Summary of clinical findings

No.	Age/ gender	Site of lesion	Symptoms	Clinical diagnosis	Atypical Ly in PB	EBV findings	Outcome
1	14/M	Epipharynx and Bil. neck LN	Sore throat	Abscess	–	VCA-IgM <10	8 m alive (–)
2	20/M	Epipharynx and Bil. neck LN	Fever	Carcinoma	NA	NA	8 m alive (–)
3	25/M	Epipharynx	–	Tumor	–	EA+, VCA-IgM × 10	12 m alive (–)
4[a]	36/M	Lt. tonsil and Lt. neck LN	–	Malignant lymphoma	9%	EA+, VCA-IgM+	24 m alive (–)
5	56/F	Rt. tonsil	–	Tumor	NE	NE	60 m alive (–)
6[b]	57/F	Lt. tonsil and neck LN	Sore throat	Malignant lymphoma	7%	VCA-IgG × 320	61 m alive (–)
7	68/F	Epipharynx and Lt. neck LN	Sore throat	Malignant lymphoma	–	VCA-IgG × 640	14 m Rt. neck LN relapse, 30 m alive (–)
8	70/M	Rt. tonsil and neck LN	Sore throat	Malignant lymphoma	–	VCA-IgG = 7.7 g/ml	48 m alive (–)
9	88/F	Rt. tonsilla	Sore throat	Tumor	–	NA	12 m died (–)

Ly in PB = Lymphocytes in peripheral blood; VCA = EBV capsid antigens; m = months; Bil. = bilateral; LN = lymph node; NA = not available; Lt. = left; Rt. = right; NE = not examined; (–) = without disease.
[a] Case 4: white blood cell count in peripheral blood 3,800/mm^3. [b] Case 6: white blood cell count in peripheral blood 5,700/mm^3.

clinical and serological findings [2]. However, a lymphoid tissue biopsy may be performed when malignant lymphoma is a clinical consideration in patients demonstrating atypical clinical features [1, 3–7]. Atypical features include age over 30 years, generalized lymphadenopathy, or isolated lymphadenopathy in an unusual site, tonsillar mass, negative heterophil antibody titer or absence of atypical lymphocytosis in peripheral blood [3–7].

In such instances, diagnostic problems for surgical pathologists may arise because the histopathological features of IM may simulate those of either Hodgkin lymphoma (HL) or non-HL [1, 3, 8, 9]. Moreover, because of the small biopsy specimens in the Waldeyer's ring (WR), differential diagnosis between EBV-associated LPD and malignant lymphoma may occasionally be difficult [6, 8]. Recently, we have reported 2 cases of tonsillar lesion of IM resembling marginal zone B cell lymphoma (MZBCL) of mucosa-associated lymphoid tissue (MALT) type [10]. To further clarify the clinicopathological findings of tonsillar lesions of IM, we reviewed 9 such cases.

Patients and Methods

Nine cases were collected from a series treated by one of the authors (M.K.) between 1999 and June 2009. The medical records of 8 cases were extensively reviewed. Five cases (No. 4–6, 8 and 9) had been reported previously [7, 10].

Specimens were fixed in formalin, routinely processed and embedded in paraffin. For light-microscopic examination, the sections were stained with hematoxylin-eosin (HE).

A basic immunohistochemical panel including antibodies against human immunoglobulin light chain (κ and λ; Novocastra, New Castle, UK or Dako A/S, Glostrup, Denmark), 4C7 (CD5; Novocastra), L26 (CD20; Dako), Ber-H2 (CD30; Dako), and DFT-1 (CD43; Dako or Novocastra) had been used in all 9 cases.

When additional slides and/or paraffin blocks were available, the immunohistochemical analysis was expanded to include antibodies 1F6 (CD4; Novocastra), 4B11 (CD8; Novocastra), C3D-1 (CD15; Dako), cocktail of 2G9 (CD21; Novocastra) and RLB25 (CD35; Novocastra), 1B12 (CD23; Novocastra), JCB117 (CD79a; Dako), P1F6 (bcl-6; Novocastra) and E29 (EMA; Dako). Immunohistochemical studies were performed using the antigen retrieval method with the avidin-biotin-peroxidase method or Ventana-automated (BenchMark™) stainer according to the manufacturer's instructions.

In situ hybridization with EBV-encoded small RNA (EBER) oligonucleotides was performed to test for the presence of EBV small RNA in formalin-fixed paraffin-embedded sections using a Ventana-automated (BenchMark) stainer or using a hybridization kit (Dako).

DNA was extracted from paraffin-embedded sections. The variable region (CDR2 and FW3) and the VDJ region (CDR3) of the immunoglobulin heavy chain (IgH) gene were amplified by seminested PCR, using primers of FR2B, LJH and VLJH, according to a previously described method [11]. Primers were as follows: 5′-CCGG(A/G)AA(A/G)(A/G)GTCTGGAGTGG-3′ as upstream consensus V region primer (FR2B); 5′-TGAGGAGACGGTGACC-3′ as a consensus J region primer (LJH), and 5′-GTGACCAGGGT [A/C/G/T] CCTTGGCCCCAG-3′ as a consensus J region primer (VLJH). PCR products were estimated to be about 200–300 bp in length.

Results

Clinical Findings

The main clinical findings are shown in table 1.

The patients including 5 men and 4 women ranged in age from 14 to 88 years with a median age of 56 years.

'B' symptoms such as fever were recorded in only 1 case (No. 2). Cervical lymph node swelling was recorded in 6 cases (No. 1, 2, 4, 6–8). Small numbers of atypical lymphocytes were found in 2 cases (No. 4 and 6). Hepatosplenomegaly was recorded in 1 case (No. 7) with chronic hepatitis virus type B infection. However, there was no atypical lymphocytosis (>10%) in peripheral blood from any of the 9 patients. Mild impairment of the liver function was demonstrated by laboratory tests in 2 patients (No. 3 and 4). Serological evidence of current or recent primary EBV infection in an initial serum specimen was established by the presence of high titers of VCA-specific IgG and antibodies in cases 6 and 7 (>×320) and in case 8 (7.7 g/ml) [2]. Positivity for early antigen was detected in cases 3 and 4. There were no abnormal serological findings for EBV in case 1. In the remaining 2 cases (No. 2 and 9), serological examinations for EBV were not performed. Information from flow cytometry was available in 3 cases (No. 6–8) that showed a polyclonal B cell population. Positive autoantibodies (direct Coombs' test and rheumatoid factor) were recorded in 1 case (No. 7). Clinically, IM was not suspected in any of the 9 cases. Malignant lymphoma was suspected in 4 cases (No. 4, 6–8), tonsillar tumor in 3 cases (No. 3, 5 and 9), carcinoma in 1 (No. 2) and tonsillar abscess in 1 (No. 1).

Follow-up information was available in all 9 patients for periods ranging from 8 to 61 months (mean 29 months, median 24 months). One female patient (No. 7) was tentatively diagnosed as having diffuse large B cell lymphoma and was given CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) therapy. Fourteen months after biopsy, bilateral neck and intra-abdominal lymphadenopathy was observed. She was diagnosed as having lymph node involvement of lymphoma and received CHOP therapy again. One patient (No. 9) died of heart failure after 12 months, while the remaining 8 cases were alive at the last follow-up.

Histological and Immunohistochemical Findings

All 9 lesions were characterized by obvious expansion of the interfollicular area and effacement of the follicles indicating atypical lymphoid proliferation. The lymphoid follicles had hyperplastic germinal centers with ill-defined borders in 5 cases (No. 3–6 and 8), although atrophic germinal centers were common in the remaining 2 cases (No. 7 and 9). There were no lymphoid follicles in two lesions (No. 1 and 2).

Three histological subtypes were delineated.

HL-Like (n = 1; Case 1)

The lesion contained numerous large lymphoid cells including mononuclear Hodgkin (H) cells with a few multinucleated Reed-Sternberg (RS)-like cells admixed with an inflammatory background (fig. 1a).

Immunohistochemical study demonstrated that large lymphoid cells including H cells and RS-like cells were CD20+ (fig. 1b), CD5–, CD15–. A portion of the large lymphoid cells including H cells and RS-like cells were CD30+. Immunohistochemical studies of light chain determinants for large lymphoid cells demonstrated a polyclonal pattern (fig. 1c, d).

T Cell/Histiocyte-Rich Large B Cell Lymphoma-Like (n = 4; Cases 2, 3, 5 and 7)

These lesions are comprised of scattered single large transformed lymphocytes embedded in a background of small to medium-sized lymphocytes and histiocytes with or without epithelioid cell features (fig. 1e). The large lymphoid cells are usually dispersed and do not form large aggregates.

The immunohistochemical study demonstrated that the majority of large lymphoid cells were CD20/79a+ (fig. 1f), whereas a minority of them were CD5+ (fig. 1g). A portion of the large B cells were CD30+. The majority of small to medium-sized lymphocytes were CD5+. There was a predominance of CD8– over CD4+ T cells in 2 cases (No. 3 and 5), whereas there was an almost equal number of CD8– and CD4+ T cells in the remaining 2 cases (No. 2 and 7). There were no CD43 and/or bcl-6+ large B cells in any of the lesions.

MZBCL of MALT Type-Like (n = 4; Cases 4, 6, 8 and 9)

The lymphoid follicles were surrounded by sheet-like proliferation of polymorphous infiltration showing a marginal zone distribution pattern (fig. 2a). Three lesions (No. 4, 8 and 9) contained numerous mature plasma cells, plasmacytoid cells and immature plasma cells with occasional immunoblasts as well as small to medium-sized lymphocytes (fig. 2b). Histiocytes with or without epithelioid cell features were seen in all 4 lesions. These epithelioid cells occasionally formed small clusters in 2 cases (No. 4 and 8). A portion of immunoblasts with large vesicular nuclei and prominent nucleoli resembled H cells in 2 cases (No. 8 and 9). However, there were no RS-like cells in any of the 4 lesions. The small lymphoid cells usually had regular round nuclei, whereas medium-sized cells occasionally showed nuclear irregularities and a moderate amount of clear cyto-

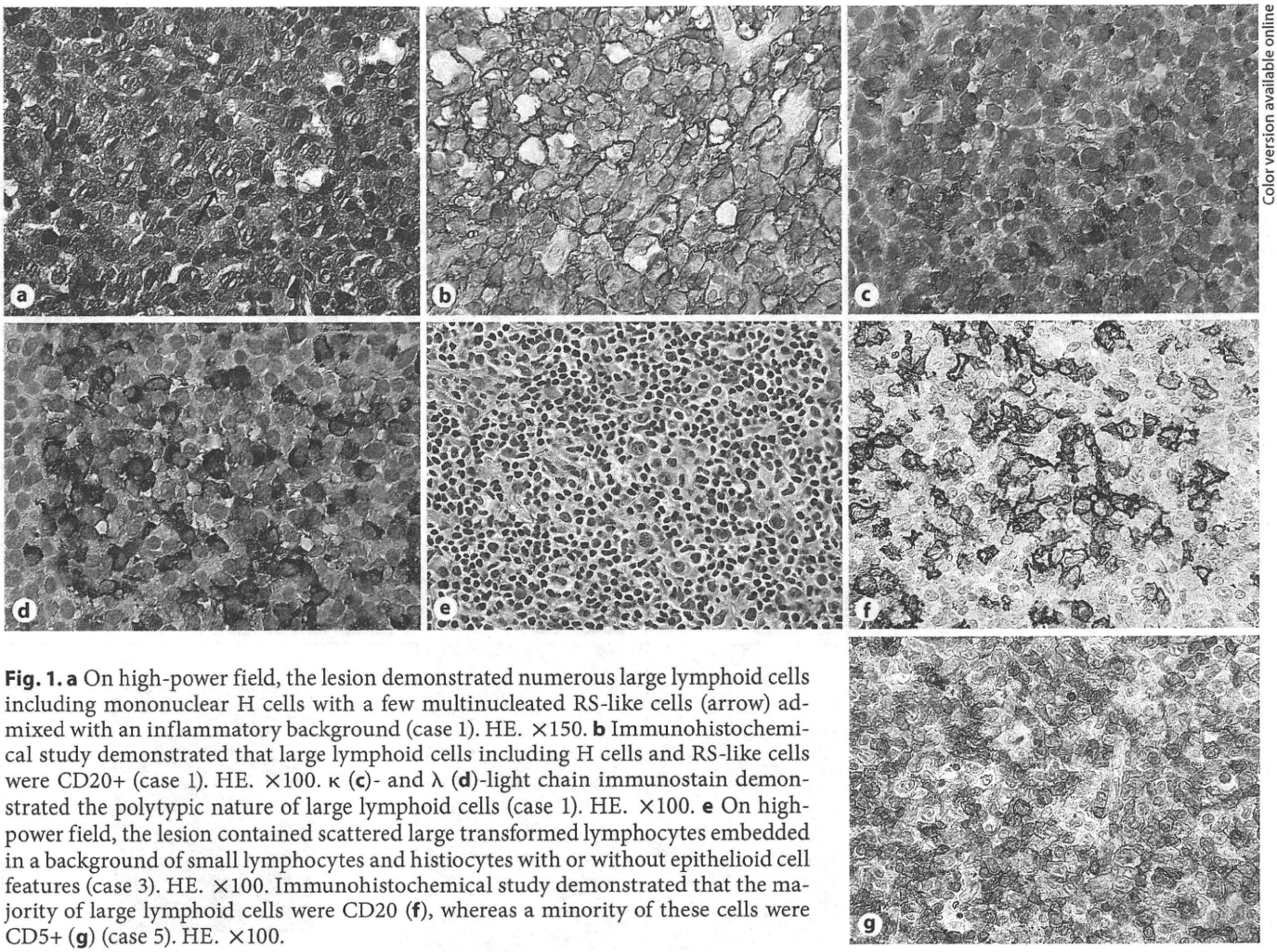


Fig. 1. a On high-power field, the lesion demonstrated numerous large lymphoid cells including mononuclear H cells with a few multinucleated RS-like cells (arrow) admixed with an inflammatory background (case 1). HE. ×150. **b** Immunohistochemical study demonstrated that large lymphoid cells including H cells and RS-like cells were CD20+ (case 1). HE. ×100. κ (**c**)- and λ (**d**)-light chain immunostain demonstrated the polytypic nature of large lymphoid cells (case 1). HE. ×100. **e** On high-power field, the lesion contained scattered large transformed lymphocytes embedded in a background of small lymphocytes and histiocytes with or without epithelioid cell features (case 3). HE. ×100. Immunohistochemical study demonstrated that the majority of large lymphoid cells were CD20 (**f**), whereas a minority of these cells were CD5+ (**g**) (case 5). HE. ×100.

plasm (fig. 2c). Lymphoepithelial lesions were not evaluated.

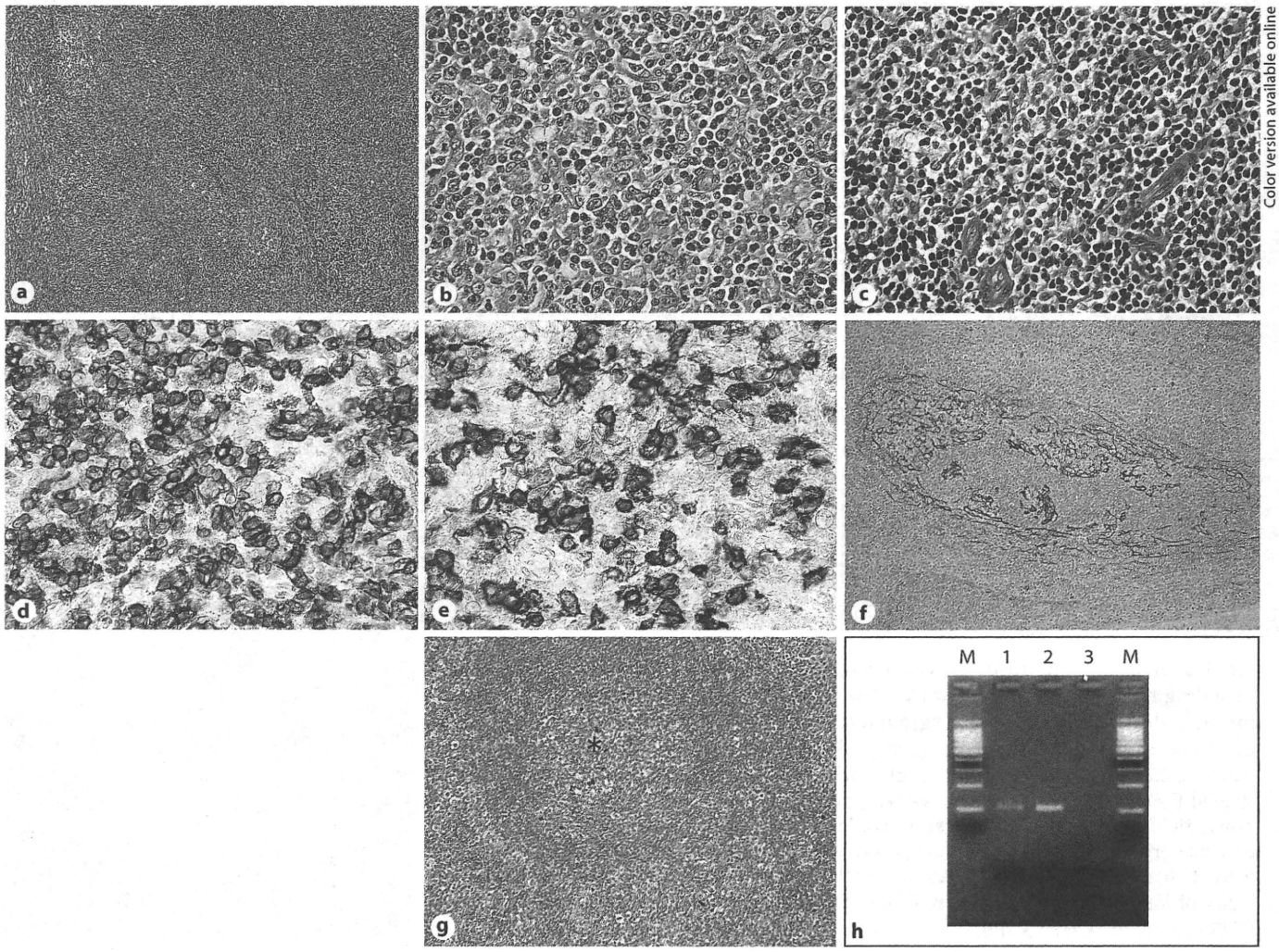
Staining with CD20 and CD5 showed the mixed nature of small and medium lymphocytes and immunoblasts including cells resembling H cells (fig. 2d, e). A portion of immunoblasts were CD30+. There were no CD43+ B lymphocytes. Immunohistochemical studies of light chain determinants for interfollicular plasma cells, plasmacytoid cells and B immunoblasts including cells resembling H cells demonstrated a polyclonal pattern. Staining with a monoclonal antibody cocktail of 2G9 + RLB25 or 1B12 highlighted the meshwork of follicular dendritic cells (FDCs). Although the majority of the FDC meshwork maintained a regular arrangement, a portion of the meshwork was broken into clusters (fig. 2f).

EBV Findings

On in situ hybridization, EBER+ cells were demonstrated in the lesions in all 9 cases. The number of EBER+ cells exceeded 200 in 6 cases (No. 1, 2, 4, 5, 8 and 9). In 1 case (No. 6), approximately 50 EBER+ cells were found. In 2 cases (No. 3 and 7), only less than 10 EBER+ cells were found in the overall lymph nodes. Scattered EBER+ cells were located in the germinal centers as well as interfollicular areas in 2 cases (No. 4 and 8) (fig. 2g), whereas EBER+ cells were located only in the interfollicular area in the remaining 7 cases (No. 1–3, 5–7 and 9).

Genotypic Findings

A discrete band of amplified IgH gene was found in 3 cases (No. 1, 6 and 8) (fig. 2h), respectively. In the remaining 4 cases (No. 2, 3, 5 and 7), only germ line bands were detected.



Color version available online

Fig. 2. a Low-power field of biopsy specimen. The lesion was characterized by diffuse interfollicular hyperplasia with small vessel proliferation and a hyperplastic germinal center with ill-defined borders (case 8). HE. ×10. **b** On high-power field, the interfollicular area contained mature plasma cells, plasmacytoid cells, immunoblasts, epithelioid histiocytes, eosinophils and small and medium-sized lymphocytes (case 8). HE. ×100. **c** On high-power field, the small lymphoid cells usually had regular round nuclei, whereas medium-sized cells occasionally showed nuclear irregularities including angulated forms and had a moderate amount of clear cytoplasm (case 6). HE. ×100. CD5 (**d**) and CD20 (**e**) immunostain demonstrated the mixed nature of the small to medium-sized lymphocytes and immunoblasts (case 8). HE. ×100. **f** A cocktail of monoclonal antibody 2G9 + RLB25 immunostaining. FDC meshwork was broken into clusters (case 8). HE. ×25. **g** Note the numerous EBV-infected lymphocytes in the germinal center (*) as well as in the interfollicular area (case 8). HE. ×25. **h** PCR analysis for clonal IgH rearrangement in case 8. The lanes contain marker (M), positive control (1), case 8 (2), negative control (3) and marker (M).

Evaluation of Biopsy Specimen of Case 7 Who Was Diagnosed as Having Diffuse Large B Cell Lymphoma

Flow-cytometric results and PCR findings in both initial and secondary biopsy specimens demonstrated a reactive nature of B lymphocytes. Recurrent lymph node biopsy specimens showed reactive monocytoid B cell hyperplasia by reexamination. Overall, this case was considered to represent essentially reactive lymphoproliferation.

Discussion

The present 9 cases demonstrated atypical clinical findings including the following: (1) all 9 cases presented with tonsillar mass; (2) 6 patients were over 30 years old; (3) absence of atypical lymphocytosis of the peripheral blood in all 9 cases; (4) presence of 'B' symptoms in only 1 case, and (5) absence of high titers of EBV antibodies in

1 case. Moreover, clinically, IM was not suspected in any of the 9 cases. Because a clinical diagnosis of malignant lymphoma could not be excluded, these 9 patients underwent tonsillar biopsy. Histologically, three types were delineated: (1) HL-like (n = 1); (2) TCHRLBCL-like (n = 4), and (3) MALT-type lymphoma-like (n = 4).

In earlier reports, the description of RS-like cells in IM generated a great deal of interest [12–14]. Consequently, the literature has tended to emphasize the differential diagnosis of IM from HL [1, 14]. However, recent reports emphasized the differential diagnostic problems between EBV-associated LPDs and non-HL particularly diffuse large B cell lymphoma [3–9].

HL occurs predominantly in a nodal-based disease, and primary extranodal presentation is very rare [8, 15]. When it presents in extranodal tissues, WR, particularly the palantine tonsil, is a common site. Most patients present with classical HL (frequently mixed cellularity subtype) and show a strong association with EBV [8, 15–17]. Case 1 contained numerous H cells and RS-like cells as well as large B cells. In case 1, the majority of the H cells and RS-like cells were CD20+, CD30+, CD15– [15]. Moreover, large B cells including H cells contained polytypic intracytoplasmic immunoglobulins [18]. These immunophenotypic findings of case 1 were different from those of classical HL [6, 15, 18].

Four of our 9 cases showed histopathological findings similar to those of TCHRLBCL [19, 20]. Histomorphologically, these 4 lesions were characterized by scattered large lymphocytes observed in a background of numerous small lymphocytes [19, 20]. The majority of the large lymphocytes showed a B cell phenotype, whereas only a minority of large lymphocytes also expressed T cell antigen. There were no bcl-6+ large B cells in any of the 4 lesions [19, 20]. Moreover, there were various numbers of CD30+ and/or EBER+ large B cells in all 4 lesions. Overall immunohistochemical and EBV findings of 4 lesions differed from those of TCHRLBCL [19, 20].

Previously, using surgically resected specimens, we have indicated that MZBCL, particularly the MALT type, frequently affected the WR [21]. Four of our 7 cases showed histopathological findings similar to those of MALT-type lymphoma. Histologically, 4 lesions were characterized by a marginal zone distribution pattern of atypical lymphocytes, which is a characteristic histological finding of MALT-type lymphoma [22, 23]. Lymphoid infiltration contained medium-sized lymphocytes with irregular nuclei and a moderate amount of clear cytoplasm somewhat resembling centrocyte-like (CCL) cells, immunoblasts, plasmacytoid cells and plasma cells [22, 23]. Plasma cell differentiation has been noted in various degrees in MALT lymphoma, and plasma cells may occasionally obscure the CCL cells [22, 23]. Moreover, a portion of the MALT-type lymphoma was associated with prominent epithelioid cell reaction [24]. Breakage of the FDC network seen in case 2 also suggested the follicular colonization of MALT-type lymphoma [22, 23]. However, cells resembling CCL cells were CD20+ but CD43– [23, 25]. The polytypic nature of the B immunoblasts, plasmacytoid cells and plasma cells was demonstrated by the immunohistochemical study. Moreover, MZBCL is rarely associated with EBV [22, 23].

Immunohistochemical and flow-cytometric study demonstrated the reactive nature of the B cells in all 9 lesions. However, 3 (No. 1, 6 and 8) of our 7 cases examined by PCR demonstrated IgH gene rearrangement. There was no development of B cell lymphoma or recurrence of B cell lymphoma in any of the 3 lesions demonstrating IgH rearrangement. Moreover, 2 (No. 6 and 8) of the 3 cases showed a relatively long-term survival (48 and 61 months). As Nam-Cha et al. [26] indicated, it remains unclear whether these cases are clinically relevant, could be a sign of the prelymphomatous stage, or merely represent an exaggeration of the normal B cell clonal response. However, 1 case (No. 1) showed a relatively short follow-up period. To clarify this issue, further study is needed.

There were no abnormal serological findings for EBV in case 1. Moreover, serological examinations for EBV were not performed in 2 cases (No. 2 and 7). In WR, reactivity of lymphoid cells for EBV has been reported in lymphoid tissues of a high percentage of patients with previously asymptomatic EBV infection [27–29]. Only a few EBV+ small lymphocytes were usually detected in patients with previously asymptomatic infection [27–29], whereas numerous EBER+ large B cells were observed in 3 lesions. These 3 cases were considered to be primary EBV infection or EBV reactivation [1, 7, 30].

When IM is not suspected clinically, microscopic findings in the tonsillar specimens can pose serious differential diagnostic problems for surgical pathologists, and may lead to a misdiagnosis of malignant lymphoma. To avoid overdiagnosis and overtreatment, we emphasize awareness of the atypical clinical presentation of EBV-related LPDs and the need for careful attention to the clinical and laboratory findings as well as morphologic features in these cases. The present study indicated that EBV-associated LPD should be added to the differential diagnosis for MZBCL.

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Distribution of IgG4- and/or IgG-Positive Plasma Cells in Hashimoto's Thyroiditis: An Immunohistochemical Study

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Key Words
Hashimoto's thyroiditis · IgG4-related sclerosing disease · Plasma cells · Immunohistochemistry

Abstract
Background and Study Aim: Recently, immunohistochemistry has shown numerous IgG4-positive plasma cells in a subset of Hashimoto's thyroiditis (HT), and this type of HT (IgG4 HT) appears to be a subtype of IgG4-related sclerosing disease. However, little is known about the distribution pattern of plasma cells in IgG4 thyroiditis. To clarify the distribution pattern of IgG4-positive plasma cells, 33 cases of HT demonstrating abundant lymphoplasmacytic infiltrate were studied. ***Methods:*** Using formalin-fixed paraffin-embedded sections, histological, immunohistochemistry and polymerase chain reaction were performed. ***Results:*** Fourteen cases were classified as IgG4 HT and 19 cases were non-IgG4 HT. Histologically, there was no significant difference between the 2 groups with regard to the degree of stromal fibrosis, lymphoid follicle formation, or the presence of phlebitis or fibrous thyroiditis. The present study demonstrated 2 distribution patterns of IgG4- and/or IgG-positive plasma cells, namely the interfollicular (n = 31) and intrafollicular + interfollicular patterns (n = 2). Interfollicular plasma cells were always polytypic intracytoplasmic immunoglobulin in all 33 cases. However, intrafollicular plasma cells in 2 lesions had monotypic kappa light chain by immunohistochemistry. ***Conclusion:*** The present study demonstrated that the majority of IgG4 HT and non-IgG HT cases showed an interfollicular distribution pattern of IgG4- and/or IgG-positive plasma cells.

Introduction

Hashimoto's thyroiditis (HT), which is characterized by the presence of goiter and serum thyroid autoantibodies, is the most common type of thyroiditis [1]. The diagnostic criteria for HT have been well described. However, HT exhibits various clinicopathological presentations and its pathogenesis is poorly understood [1]. IgG4-related sclerosing disease is a recently recognized entity, clinically characterized by mass-forming lesions in the exocrine glands, extranodal organs (most frequently pancreas, biliary tract, salivary glands and lacrimal gland) and

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lymph nodes as well as elevated serum IgG4 level, and shows a good response to steroid therapy [2, 3]. It is characterized by prominent lymphoplasmacytic infiltration and sclerosis as well as numerous IgG4-positive plasma cell infiltrations [3]. The fibrous variant of HT is characterized by a marked fibrous replacement of one third or more of the thyroid parenchyma [4, 5]. Harach and Williams [6] found that the fibrous variant of HT contained numerous IgG-positive plasma cells. In 2005, Komatsu et al. [7] demonstrated a high prevalence of hypothyroidism among patients with autoimmune pancreatitis. Histopathological and immunohistochemical findings of the fibrous variant of HT are similar to those of IgG4-related disease. Recently, Li et al. [8, 9] demonstrated that from both clinical and histopathological [i.e. presence of prominent fibrosis, numerous IgG4-positive plasma cells and elevated serum IgG4 level (>135 mg/dl)] perspectives, IgG4 HT and non-IgG4 HT appear as distinct entities [3]. They emphasized that measuring the serum IgG4 level provided a useful method of distinguishing IgG4 HT from non-IgG4 HT [9]. In IgG4-related lymphadenopathy, Sato et al. [10] classified two types of IgG4-related lymphadenopathy by the infiltration pattern of IgG4-positive plasma cells: interfollicular plasmacytosis and intragerminal center plasmacytosis. However, little is known about the distribution pattern of IgG4-positive plasma cells in IgG4 HT. To clarify the presence or absence of 2 types of IgG4-positive plasma cell infiltration in IgG4 HT, we studied 33 cases of HT demonstrating prominent lymphoplasmacytic infiltration in comparison with non-IgG4 HT.

Patients and Methods

Thirty-three patients with HT who underwent total thyroidectomy at Kuma Hospital (Kobe, Japan) between 1983 and 2006 were reviewed. According to the Guidelines of the Japanese Thyroid Society, all patients were diagnosed as having HT based on clinical findings: diffuse swelling of the thyroid gland without any other cause (such as Graves' disease) accompanied by any one of the following laboratory findings: (1) positive for antithyroid microsomal antibody or antithyroid peroxidase antibody, (2) positive for antithyroglobulin antibody, and (3) lymphocytic infiltration in the thyroid gland confirmed with cytological examination. Incidental findings of focal (nonspecific) lymphocytic thyroiditis in tumor-bearing thyroid tissue were excluded from this study. This study was approved by the Kuma Hospital Bioethical Committee.

Surgical specimens were fixed in formalin, routinely processed and embedded in paraffin. For light-microscopic examination, the sections were stained with hematoxylin-eosin (HE) and Victoria blue-HE stain.

Immunohistochemical studies were performed using automated Histofine Histostainer (Nichirei Bioscience Inc., Tokyo, Japan) according to the manufacturer's instructions.

The panel of antibodies included human immunoglobulin light chains (kappa and lambda; Dako A/S, Glostrup, Denmark), IgA (Dako), IgG (Dako), MCO011 (IgG4; Binding Site, Birmingham, UK), IgM (Dako) and a cocktail of 2G9 (CD21; Novocastra) and RB L25 (CD35; Novocastra). If necessary, polyclonal CD3 (Dako), 56C6 L26 (CD20; Dako), DFT-1 (CD43; Dako), 124 (bcl-2; Dako), AE1/3 (cytokeratin; Dako) and antithyroglobulin antibody (Dako) were also stained. Sections with known reactivity for the antibodies assayed served as positive controls and sections treated with normal rabbit and mouse serum served as negative controls.

In selected cases, genomic DNA was extracted from formalin-fixed tissues after dewaxing of paraffin sections; then immunoglobulin heavy chain (IgH) rearrangement was analyzed by polymerase chain reaction as described previously [11].

IgG4 HT was defined by the criteria of Li et al. [8, 9], i.e. (1) greater than 20 IgG4-positive plasma cells/high power field and (2) greater than 30% IgG4/IgG ratio. The degree of stromal fibrosis and lymphoid follicle formation was examined and expressed as 3+ = severe, 2+ = moderate, and 1+ = mild.

Comparison of frequency data between the 2 groups was performed using the Fisher exact test. For continuous data, intergroup comparisons were performed using the Mann-Whitney U test.

Results

Fourteen cases were classified as IgG4 HT and the remaining 19 were classified as non-IgG4 HT.

Clinical Findings

Main clinical data of IgG4 HT and non-IgG4 HT patients analyzed in the study including age, gender, weight of resected thyroid gland, positivity for antithyroid gland antibodies, thyroid function and presence or absence of polyclonal hypergammaglobulinemia were collected and summarized in table 1. There was a greater proportion of males in the IgG4 HT group, but the difference was not significant. In comparison with non-IgG4 HT, patients with IgG4 HT demonstrated a high incidence of hypothyroidism ($p < 0.005$).

Pathological and Immunohistochemical Findings

The main pathological findings are summarized in table 2.

Histopathologically, both groups were characterized by follicular cell degeneration, dense lymphoplasmacytic infiltration and various degrees of lymphoid follicle formation and stromal fibrosis (fig.1a, b). Obstructive phlebitis was observed in both groups (IgG4 HT = 2, non-

Table 1. Summary of clinical findings in 33 cases

	IgG4 HT	Non-IgG4 HT
Number of cases	14	19
Gender (m:f)	4:10	2:17
Age[1], years	41–66 (54, 56)	44–66 (56, 54)
Weight of thyroid gland[1], g	77–371	65–367 (190, 203)
Thyroid test (+)	8/12	7/18
Microsome test (+)	11/12	10/17
Hypothyroid	12	9
Hypergammaglobulinemia (>20%)	8/9	10/13

[1] Values represent mean range with the median given in parentheses.

Table 2. Summary of pathological findings in 33 cases

	IgG4 HT	Non-IgG4 HT
Number of cases	14	19
Stromal fibrosis (3/2/1)	10/3/1	8/4/7
Lymphoid follicles (3/2/1)	1/10/3	5/9/5
Obstructive phlebitis	2	4
Fibrous thyroiditis[1]	4	2
Presence of numerous plasma cells in germinal center	2	1
Presence of numerous IgG4+ plasma cells in germinal center	1	0

[1] Fibrous replacement of one third or more of the thyroid parenchyma [3, 4].

IgG4 HT = 4) (fig. 1c). There were numerous mature plasma cells in a portion of the lymphoid follicles in 2 cases (IgG4 HT = 1, non-IgG4 HT = 1) (fig. 1d). Four cases (IgG4 HT = 4, non-IgG4-HT = 2) were diagnosed as fibrous thyroiditis because there was fibrous replacement of one third or more of the thyroid parenchyma [4, 5].

There was a greater proportion of fibrous thyroiditis in IgG4 HT. However, there was no significant difference between the 2 groups with regard to the stromal fibrosis (moderate/severe), lymphoid follicle formation (moderate/severe), or presence of obstructive phlebitis or fibrous thyroiditis.

An immunohistochemical study of the light chain determinant of intracytoplasmic immunoglobulins demonstrated a polytypic nature in the interfollicular area in all 33 lesions (kappa/lambda ratio approximately 3–4/1), whereas in the 2 lesions containing numerous plasma cells in the germinal center, intrafollicular plasma cells demonstrated marked kappa light chain predominance (kappa/lambda ratio was higher than 10/1) (fig. 2a, b). Intragerminal center plasma cells demonstrated a monotypic population based on the criteria of Lennert and Feller [12].

There were numerous IgG-positive plasma cells with scattered IgA- or IgM-positive plasma cells in all 33 cases. In 31 cases (IgG4 HT = 13, non-IgG4 HT = 1), IgG4- and/or IgG-positive plasma cells were mainly located in the interfollicular area (fig. 2c, d) (interfollicular pattern), whereas in 2 lesions containing kappa-light-restricted plasma cells in the germinal centers, the majority of plasma cells in the germinal center as well as in the interfollicular area were also IgG4- and/or IgG-positive (fig. 2e) (intrafollicular + interfollicular pattern). Thyroglobulin was detected in the cocktail of CD21 and CD35-antibody-positive follicular dendritic cell networks in the germinal centers as well as in the follicular epithelium in the cases examined (IgG4+ = 9, IgG4 = 6) (fig. 2f).

Clinicopathological, Immunohistochemical and Genotypic Analysis of the Two Cases Showing an Intrafollicular + Interfollicular Pattern

Unfortunately, information on staging was incomplete in the present series because the initial pathological diagnosis was HT. In both cases, adequate follow-up data were not available.

Histologically, there were no Dutcher bodies (intranuclear inclusions), centrocyte-like (CCL) cells or amyloid deposition. Staining for CD20 and CD3 showed the mixed nature of small lymphocytes. There were no CD20- and CD43-positive CCL cells. There were not even lymphoepithelial lesions detected by immunostaining for cytokeratin in both lesions. Polymerase chain reaction analysis demonstrated that there was no clonal rearrangement of the immunoglobulin heavy chain.

Discussion

We demonstrated 2 distribution patterns of IgG4- and/or IgG-positive plasma cells in IgG4 HT and non-IgG4 HT as well as in the lymph node lesion of IgG4-related sclerosing disease [10], i.e. the interfollicular pattern and the intrafollicular + interfollicular pattern. Interestingly, 1 case each of IgG4 HT and non-IgG4 HT showing