of Diff-Quik stain™ (Dade Behring, Newark, DE, USA). The pellets in the BALF were analyzed for lymphocyte subsets by flow cytometry at the Special Reference Laboratory).

Statistics

All results are reported as means ± standard deviation (SD), with the P-MD and L-MD groups compared using the Mann–Whitney *U*-test or Fisher's exact test, as appropriate. *P* values of <0.05 were considered statistically significant.

Results

Patient profiles

Patient profiles are summarized in Table 1. Ages at the time of diagnosis ranged from 24 to 80 years. with a mean of 60 ± 15 years. Of the 25 patients, 11 (44.0%) had a history of allergic rhinitis or sinusitis, and 7 (28.0%) had bronchial asthma. Allergic rhinitis (46.2 vs. 36.3%) and bronchial asthma (38.5 vs. 9.0%) were more common in the P-MD than in the L-MD group. Thirteen patients (52.0%) visited the hospital because of swelling of the lacrimal, parotid, and/or submandibular glands, and 7 (28.0%) complained of respiratory problems such as cough or dyspnea or had abnormal findings on chest X-ray.

Details of the 13 patients in the P-MD group are shown in Table 2. At diagnosis of MD, 7 (53.8%) of these patients had extra-pulmonary lesions, including 3 with AIP (patients 3, 4, and 13 in Table 2), 1 with retroperitoneal fibrosis (patient 2), 3 with interstitial nephritis (patients 4, 8, and 12), and 1 with hypophysitis (patient 5).

Laboratory findings

All 25 patients had abnormally high serum concentrations of total protein, IgG, and IgG4, with mean concentrations of 8.4 g/dl, 2872 mg/dl, and 926 mg/dl, respectively. In addition, 21 patients (84%) had high serum IgE concentrations, averaging 897 IU/ml. IgA and IgM concentrations, however, were within normal ranges in all patients. All patients were negative for anti-SS-A and anti-SS-B antibodies, but 6 (24%) and 7 (28%) were positive for rheumatoid factor (RF) and antinuclear antibodies (ANA), respectively. Laboratory results in the P-MD and L-MD groups are summarized in Table 3.

Serum total protein, IgG, IgG4, and sIL-2R concentrations were each significantly higher, and CH50 was significantly lower, in the P-MD than in the L-MD group. The 6 patients positive for immune complexes were all in the P-MD group. IgE concentrations did not differ significantly between the P-MD and L-MD groups.

Imaging analysis

Chest CT images of PM-D patients showed various abnormalities in the bronchi, bronchovascular bundles, alveolar septa, pleura, and hilar lymph nodes (Table 4; Fig. 1). CT showed thickening of the bronchial wall in 8 patients (61.5%, Fig. 1a), consolidation in 4 (30.8%, Fig. 1b), nodule(s) in 4 (30.8%, Fig. 1c), ground glass opacity in 3 (23.1%), interlobular thickening in 2 (15.4%), pleural thickening and/or effusion in 3 (23.1%), and mediastinal lymphadenopathy in 10 (76.9%, Fig. 1d), with many patients showing more than one of these findings. Thickening of the bronchial wall was observed in all five patients with asthmatic symptoms.

Table 1 Clinical and demographic characteristics of patients with Mikulicz's disease

	All (n = 25)	P-MD (n = 13)	L-MD (n = 11)
Age (years)	60 ± 15	61 ± 17	63 ± 9
Sex	M 18:F 7	M 11:F 2	M 6:F 5
Allergic history	11 (44.0%)	7 (53.8%)	4 (36.3%)
Rhinitis/sinusitis	9 (36.0%)	6 (46.2%)	3 (27.3%)
Bronchial asthma	7 (28.0%)	5 (38.5%)	1 (9.0%)
Both of them	5 (20.0%)	4 (30.1%)	1 (9.0%)
Chief complaint			
Glandular swelling	13 (52.0%)	3 (23.1%)	10 (91.0%)
Cough	5 (20.0%)	5 (38.5%)	
Dyspnea	1 (4.0%)	1 (7.7%)	
Abnormal chest X-ray	1 (4.0%)	1 (7.7%)	
Others	5 (20.0%)	3 (23.1%)	1 (9.0%)

P-MD Mikulicz's disease with pulmonary and/or mediastinal lesions, *L-MD* Mikulicz's disease limited to the lacrimal and/or salivary glands

Table 2 Clinical features of 13 MD patients with pulmonary lesions

	Age (years)	Sex	Extra-pulmonary lesions	IgG (mg/dl)	IgG4 (mg/dl)	IgE (IU/ml)	CH50 (U/ml)	Chest CT findings	Histological findings of pulmonary lesions	BALF		
										TCC	Lym (%)	CD4/8
1	71	F		2386	615	2585	34	Bronchial thickening	Chronic bronchitis, IgG <10/HPF	1.5	12.4	0.9
2	69	M	RPF	2731	269	975	14	Small nodules				
3	25	M	AIP	4859	3405	1100	13	Diffuse reticular shadows, consolidation, bronchial thickening	Lymphoplasmacytic infiltration IgG4/IgG >50%	2.3	20	0.6
4	41	M	AIP, nephritis	6361	776	1123	17	Diffuse reticular shadows, bronchial thickening				
5	75	M	Hypophysitis	2373	241	1868	28	Multiple masses	Inflammatory pseudotumor IgG4/IgG >40%	1.2	24.9	2.5
6	75	M		8361	4075	44	37	Focal infiltration	Interstitial lymphoplasmacytic infiltrate, IgG4 cells <10/HPF	1.2	41	2.2
7	58	M		4307	2490	223	34	Pleural effusion, consolidation				
8	74	M	Nephritis	4387	1320	560	5	Hilar lymphadenopathy				
9	35	M		1617	485	108	44	Hilar lymphadenopathy				
10	69	M		1955	991	853	37	Bronchial thickening	Chronic bronchitis, IgG4/G >50%	1.6	23.4	2.6
11	71	M		5117	384	1141	7	Hilar lymphadenopathy, pleural thickening	Chronic bronchitis, IgG4/G >50%	2.7	5.8	0.9
12	66	F	Nephritis	4174	1260	100	35	Interstitial lobular thickening, bronchial thickening, pleural thickening	Chronic bronchitis, IgG4/G >50%	4.8	18.5	1.6
13	59	M	AIP	2456	1850	1160	29	Bronchial thickening				

BALF bronchoalveolar lavage fluid *CT* computed tomography *HPF* high-power field, *TCC* total cell count ($\times 10^5$ cells/ml), *RPF* retroperitoneal fibrosis, *AIP* autoimmune pancreatitis, *Lym* lymphocytes

Table 3 Laboratory findings in the P-MD and L-MD groups

	P-MD ($n = 13$)	L-MD ($n = 11$)	P
Age (years)	61 ± 17	58 ± 14	
Sex (male/female)	13/2	6/5	0.193
Total protein (g/dl)	9.1 ± 1.8	7.5 ± 0.5	0.006
Serum IgG (mg/dl)	3930 ± 1956	1716 ± 434	<0.001
Serum IgG4 (mg/dl)	1347 ± 1227	428 ± 197	0.008
CH50 (U/ml)	25.7 ± 12.9	43.4 ± 7.2	0.003
IgE (IU/ml)	842 ± 757	1010 ± 1039	0.160
sIL-2R (U/ml)	1625 ± 1195	525 ± 238	<0.001
Immune complex	63.7%	0%	0.013
RF	30.7%	18.2%	0.640
ANA	33.3%	9.1%	0.317

sIL-2R soluble interleukin-2 receptor, *RF* rheumatoid factor, *ANA* antinuclear antibodies

Table 4 Chest CT findings

	$n = 13$
Bronchial wall thickening	8 (61.5%)
Consolidation	4 (30.8%)
Nodule/mass	4 (30.8%)
Ground glass opacity	3 (23.1%)
Interlobular thickening	2 (15.4%)
Pleural thickening/effusion	3 (23.1%)
Lymphadenopathy	10 (76.9%)

Ga-67 scintigraphy was performed in 11 P-MD patients and FDG-PET in 2. Of these 13 patients, 10 (76.9%) showed Ga-67 or 18F-FDG accumulation in mediastinal lymph nodes, 2 (15.4%) showed diffuse Ga-67 accumulation in bilateral lung fields (patients 3 and 4), and 2 (15.4%) showed patchy accumulation in the lesions (patients 5 and 12).

Histopathological findings

Histological examination of the bronchus and/or lung was performed in 7 patients, all of whom showed numerous lymphoplasmacytic infiltrates in the bronchial mucosa, peri-bronchovascular bundles (Fig. 2a), and alveolar septa. We also observed invasion by IgG4-positive plasma cells (Fig. 2b). Typical sclerosing inflammation was found in only one patient, who had an inflammatory pseudotumor (patient 5, Fig. 2c, d).

BALF findings

BALF was analyzed in 7 patients in the PM-D group. The mean total cell count was somewhat high ($2.2 \pm 1.3 \times 10^5$/ml) with a lymphocyte predominance (20.9 ± 11.1%), but there was no increase in the CD4/CD8 lymphocyte ratio (1.61 ± 0.8).

Clinical outcomes

Of the 25 patients, 17 (11 PM-D, 5 L-MD, and 1 MD with arthritis) were treated with prednisolone (initial dose 20–50 mg/day), with all rapidly responding to steroid therapy. Three years after the initiation of the steroid therapy, however, one patient (patient 3) experienced a recurrence as an intra-orbital tumor during maintenance therapy (7.5 mg/day). One other patient (the one who had MD with arthritis) developed arthropathy 8 years after the initiation of the steroid therapy, and was treated with prednisolone (10 mg/day) and salazosulfapyridine (2 g/day) [22].

Discussion

After the first description of the relationship between MD and IgG4 in 2004 [5], IgG4-related MD, also called IgG4-positive multi-organ lymphoproliferative disorder, was found to be a systemic disease accompanied by coexisting autoimmune pancreatitis (AIP), interstitial nephritis, and interstitial pneumonia [7]. MD has now been recognized as a representative feature of IgG4-related disease.

Although respiratory involvement has been reported in IgG4-related disease, most patients have been described as having complications of AIP [23–26]. As IgG4-related disease can present with various conditions in various organs, we focused on respiratory involvement in patients with IgG4-related MD.

We found that 44% of our patients with IgG4-MD had a history of allergic rhinitis and/or bronchial asthma, and that 28% complained of respiratory symptoms. In comparison, the incidence of allergic rhinitis in the general population

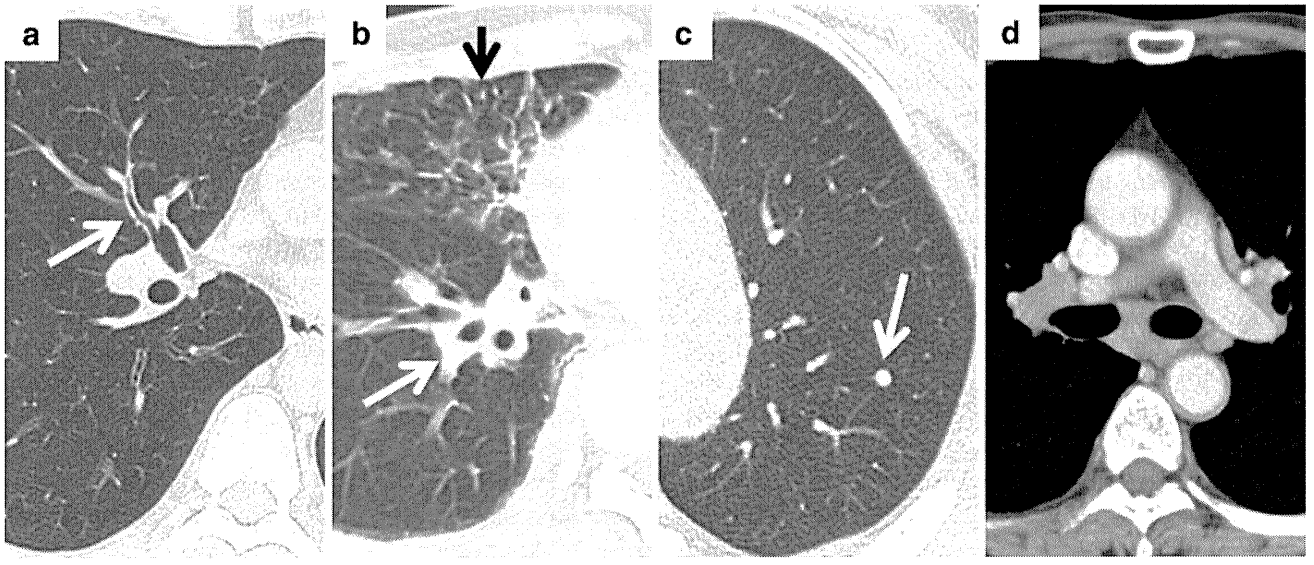

Fig. 1 Chest computed tomography (CT) findings, showing **a** bronchial thickening (*white arrow*) in the right upper lobe; **b** interlobular thickening (*black arrow*) in the right middle lobe, and thickening of the bronchovascular bundle (*white arrow*) in the right lower lobe; **c** a solid small nodule (*white arrow*) in the left upper lobe; and **d** mediastinal lymphadenopathy

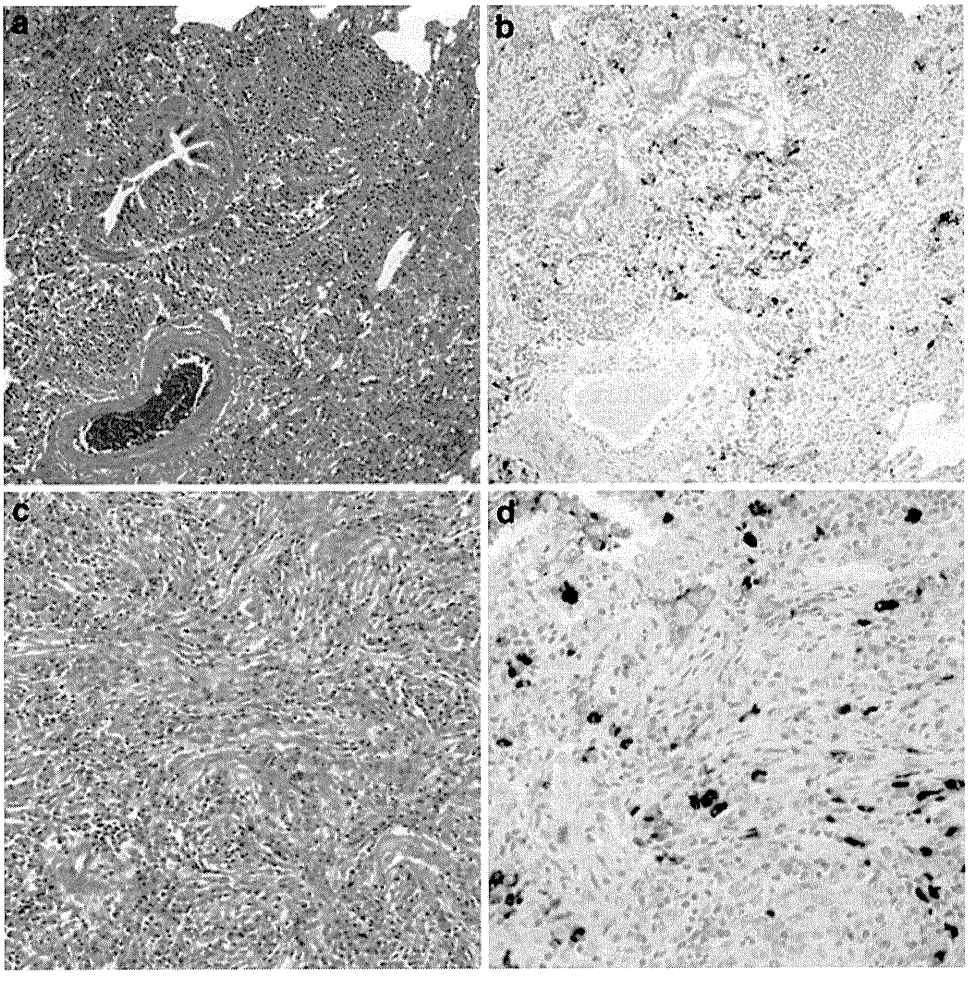

Fig. 2 Histopathology of pulmonary lesions, showing **a** peri-bronchial inflammation with lymphocytes (H&E, ×100 original magnification); **b** increased numbers of IgG4-positive plasma cells among the inflammatory infiltrates (IgG4, ×100); **c** fibrosis with a storiform pattern (H&E, ×200); and **d** infiltrates of IgG4-positive plasma cells among proliferating fibroblasts. (IgG4, ×200)

of Japanese aged around 60 years, including individuals with cedar pollinosis, is about 20–30% [27]. Thus, the frequency of allergic symptoms such as rhinitis and asthma in patients with MD, especially those with P-MD, was higher than that in the general Japanese population.

Allergic rhinitis has been found to be more common in patients with MD than in those with typical Sjögren's syndrome (40.6 vs. 14.1%) [7]. The reason for the higher incidence of allergy is unclear, but the expression of Th2 cytokines and regulatory cytokines was found to be upregulated in lesions containing IgG4-positive plasma cell infiltrates [9]. Th2 cytokines play important roles in the pathogenesis of allergic disorders such as allergic rhinitis and bronchial asthma, suggesting that the pathogenesis of allergic disorders and that of IgG4-related diseases are closely related [28].

We found that most of our patients with IgG4-MD had elevated serum concentrations of total protein, IgG, IgG4, and IgE, and that patients with P-MD showed significantly higher concentrations of total protein, IgG, IgG4, and sIL-2R than those with L-MD. Moreover, patients with P-MD tended to show disorders in many organs and had hypocomplementemia. The mean serum IgG and IgG4 concentrations in patients with AIP and pulmonary involvement have been reported to be 2747 and 1185 mg/dl, respectively [25]. Hypocomplementemia has been observed in 17–36% of patients with AIP and has been regarded as being associated with disease activity [29]. These reports and our results suggest that disease activity is high in patients with MD or AIP with pulmonary involvement, and that pulmonary involvement may be associated with disorders of other organs. Conversely, the combination of hypergammaglobulinemia and hypocomplementemia may indicate multi-organ involvement, including pulmonary lesions, in MD. Careful investigations are necessary to diagnose conditions associated with IgG4-related disease.

Of our 25 patients, 13 (52%) had IgG4-related abnormalities on chest CT, including abnormalities in the bronchi, bronchovascular bundles, alveolar septa, pleura, and hilar lymph nodes. Findings of hilar and mediastinal lymphadenopathy on CT were supported by Ga-67and/or FDG-PET accumulation. Lung lesions have been reported in 51.2% of patients with AIP, with hilar lymphadenopathy in 78.3% [30]. Our findings suggest that Ga-67 scintigraphy and FDG-PET may be useful tools for detecting the presence and distribution of these lesions [31]. As their radiologic features and distributions resembled those of sarcoidosis [25, 32], we measured serum angiotensin-converting enzyme (ACE) concentrations in our P-MD group and serum IgG4 in 13 patients with sarcoidosis. We found that the mean serum ACE concentration in the P-MD group was 13.8 ± 4.2 IU/ml, within the normal range of 7.0–25.0 IU/ml, and the mean concentration of serum IgG4 in the sarcoidosis patients was 41.2 ± 23.2 mg/dl, also within the normal range. BALF in the P-MD group showed lymphocyte predominance, but, in contrast to the findings in patients with sarcoidosis, the CD4/CD8 lymphocyte ratio was not altered. Measurements of serum ACE and IgG4 concentrations and CD4/CD8 in BALF should be sufficient to distinguish IgG4-MD from sarcoidosis when either disease is suspected on chest CT or radioisotope images.

Histopathological examination of transbronchial or surgical lung biopsy samples showed infiltrates of lymphocytes and plasma cells in the bronchial mucosa and peri-bronchovascular bundles. Findings characteristic of AIP, such as obliterative phlebitis and storiform fibrosis, were seen only in the 1 patient (patient 5) with inflammatory pseudotumor of the lung. A study of 21 patients with IgG4-related lung and pleural disease showed that irregular fibrosis and obliterative vascular changes were observed in almost all the solid nodules and pleural lesions [33]. Although two of our patients (patients 1 and 6) showed chest CT abnormalities with abundant infiltration of IgG4-positive plasma cells in their salivary glands, biopsy samples of the respiratory apparatus showed few IgG4-positive plasma cells (<10 cells per high-power field [HPF], with each having a mean of 8 cells per HPF) among many lymphocytes with tissue fibrosis. One of these patients (patient 1) had received short-term oral steroid therapy for bronchial asthma 1 month before bronchial biopsy, suggesting that short-term steroid therapy had removed IgG4-positive plasma cells from the bronchial mucosa. In the other patient (patient 6), the infiltrative shadow on CT disappeared spontaneously within a few weeks after transbronchial lung biopsy. Spontaneous remission of lesions has been observed in patients with IgG4-related diseases. Patients with AIP and duodenal papilla negative for IgG4 (mean, 6.6 cells per HPF, range 0–17 cells per HPF) appeared to have a high frequency of remission without steroid therapy [34], suggesting that our patient 6 may have presented with the same clinical condition (i.e., AIP and duodenal papilla negative for IgG4).

Corticosteroid therapy has been regarded as effective for IgG4-related disease [35, 36]. Although all of our patients who received steroid therapy responded to treatment, 2 developed either extra-glandular or extra-pulmonary lesions several years after the initiation of the steroid therapy.

To our knowledge, this report is the first to describe respiratory involvement associated with IgG4-MD. We found that almost 50% of IgG4-MD patients presented with abnormal findings on chest CT, and that IgG4-MD patients with pulmonary disorders frequently had allergic symptoms and presented with marked hypergammaglobulinemia

when compared with IgG4-MD patients with lesions limited to the lacrimal and/or salivary glands. Furthermore, the initial clinical manifestations of pulmonary disease differed in patients with IgG4-MD and those with AIP, although imaging and laboratory findings were the same. Pulmonary physicians should include IgG4-related disease in the differential diagnosis of asthmatic patients with hypergammaglobulinemia. Rheumatologists and hematologists should perform tests for respiratory lesions when patients with MD present with persistent cough.

In conclusion, pulmonary involvement associated with IgG4-MD is not rare. The careful diagnosis of IgG4-related MD should exclude other similar diseases, such as sarcoidosis, bronchial asthma, Castleman's disease, Sjögren's syndrome, and malignant lymphoma. As IgG4-related disease is still a developing entity, the details of each organ condition should be clarified.

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Conflict of interest None.

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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

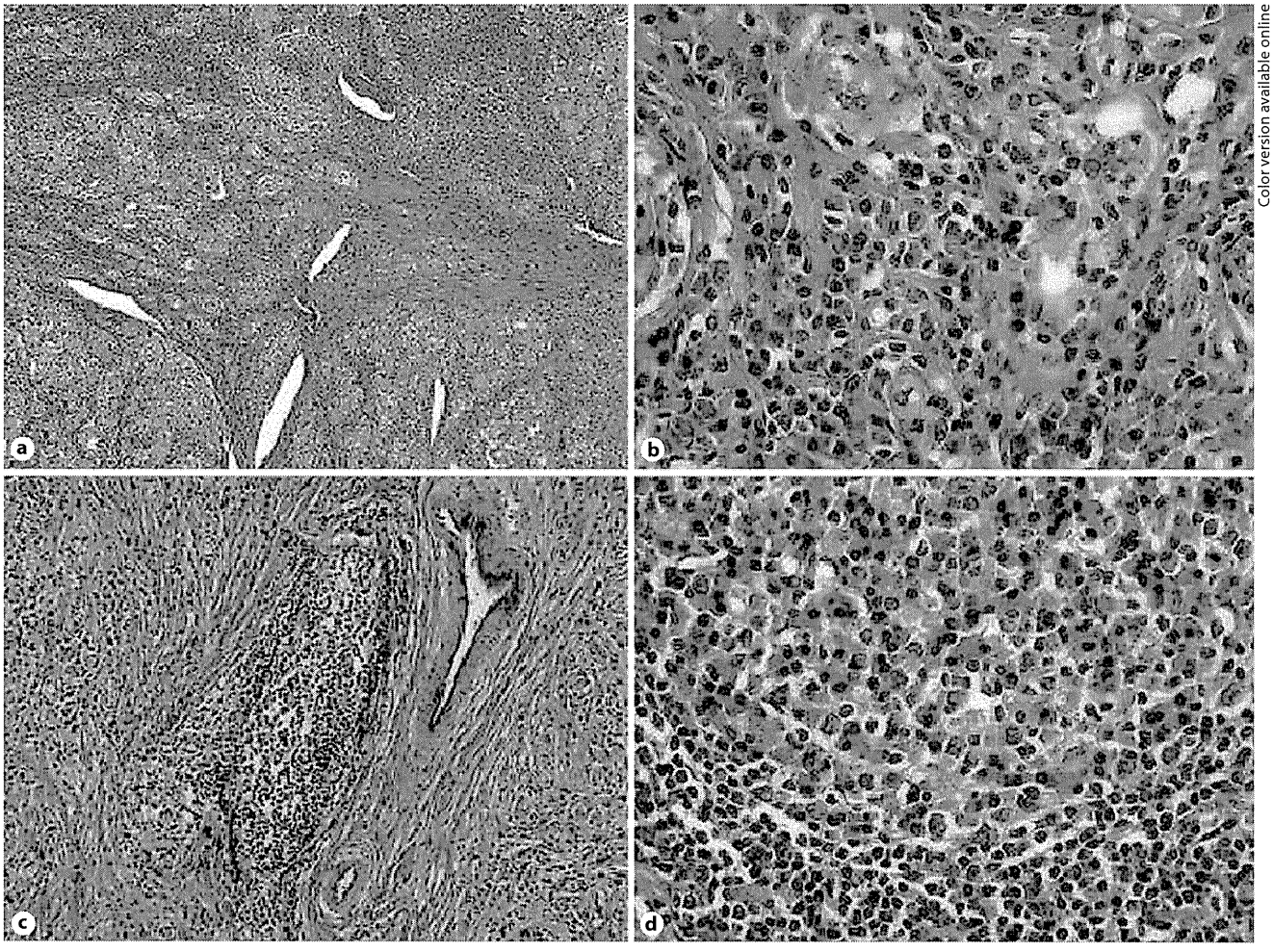

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Fig. 1. a On a low-power field, the lesion was characterized by dense inflammatory processes, various degrees of lymphoid follicle formation and stromal fibrosis. HE. ×4. **b** On a high-power field, there were prominent lymphoplasmacytic infiltrations associated with follicular cell degeneration. HE. ×40. **c** On a medium-power field, Victoria blue-HE stain demonstrated obstructive phlebitis. ×20. **d** On a high-power field, numerous plasma cells were seen in the germinal center surrounded by mantle cells. HE. ×40.

the intrafollicular + interfollicular pattern demonstrated kappa light chain-restricted germinal centers. These histological and immunohistological findings are similar to those of follicular colonization of extranodal marginal zone B cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type showing prominent plasma cell differentiation [13]. In comparison with other types of MALT-type lymphoma, plasma cell differentiation is more prominent in thyroid MALT-type lymphoma [14]. Moreover, the majority of thyroid MALT-type lymphomas demonstrated an intracytoplasmic kappa light chain [15]. However, there were no CD20- and CD43-positive CCL cells or lymphoepithelial lesions in either case [14, 16]. Using a cocktail of CD21 and CD35 antibodies, there was no broken follicular dendritic cell network, which is a characteristic immunohistochemical finding of follicular colonization of MALT-type lymphoma in either of the 2 lesions [14, 16]. A few cases of IgG4-producing MALT-type lymphoma have been reported [17]. However, overall these immunohistochemical findings suggested that these 2 lesions differed from MALT-type lymphoma.

Riedel's thyroiditis has commonly been reported as a thyroid involvement of IgG4-related sclerosing disease by some researchers [18]. In this study, a small number

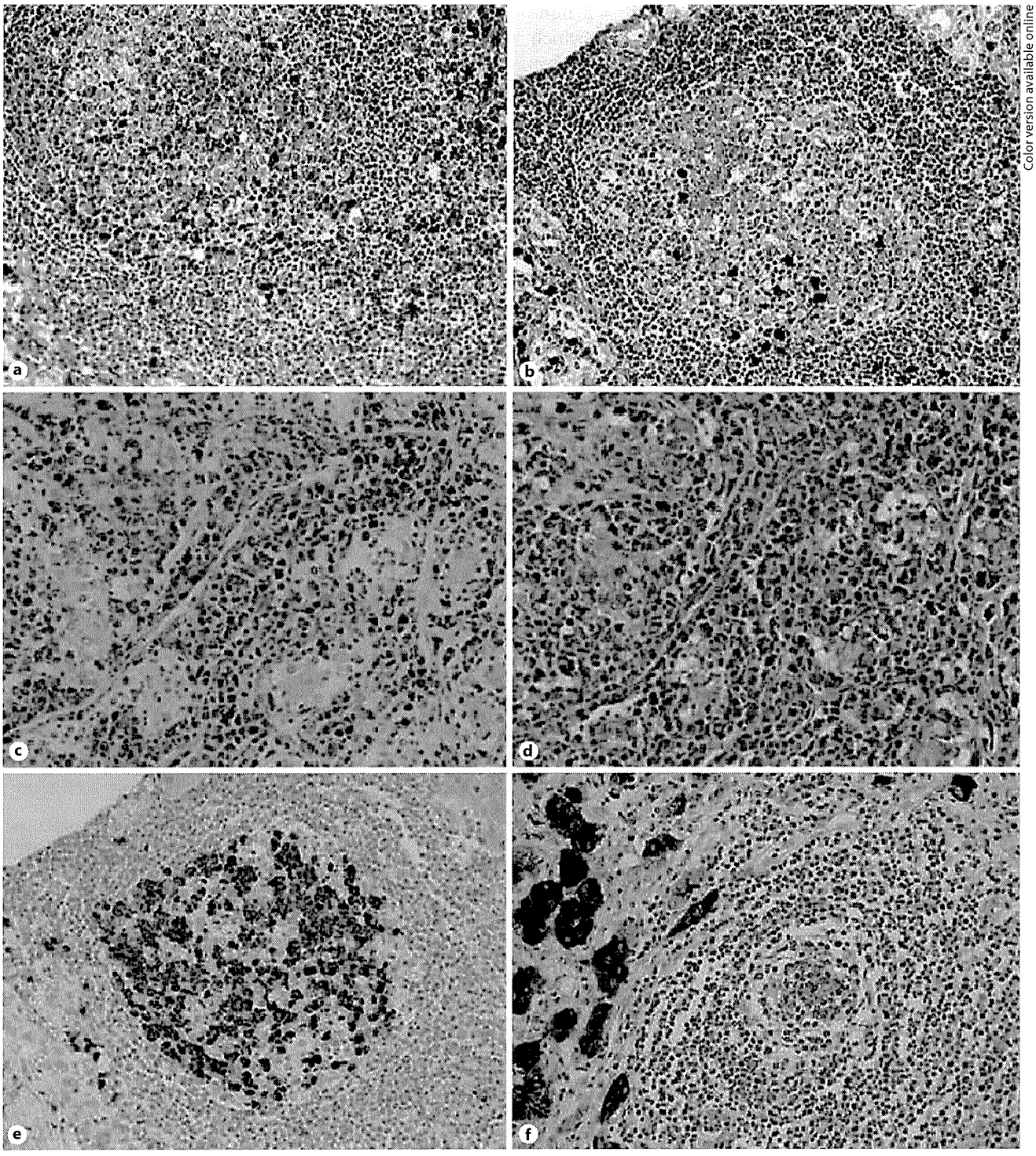

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Fig. 2. In an immunohistochemical study, intrafollicular plasma cells demonstrated marked kappa light chain predominance (kappa/lambda ratio was greater than 10/1): kappa (**a**) and lambda (**b**). Same case as in figure 1c. ×20. IgG4-positive plasma cells comprised more than 30% of IgG-positive plasma cells in the interfollicular area: IgG4 (**c**) and IgG (**d**). ×20. **e** Note numerous IgG4-positive plasma cells in the germinal center. ×20. Same case as figure 1c. **f** Thyroglobulin was detected by a cocktail of CD21 and CD35 antibody-positive follicular dendritic cell networks in germinal centers as well as in follicular epithelium. ×10.

of IgG4 HT and non-IgG4 HT cases (IgG4 HT = 2, non-IgG4 HT = 4) demonstrated obstructive phlebitis which is a characteristic histological finding of Riedel's thyroiditis and IgG4-related sclerosing disease [3–5, 19]. However, there was no extrathyroid fibrosis, which is another characteristic histological finding of Riedel's thyroiditis [3, 5]. Thyroglobulin was detected at the follicular dendritic cell networks in germinal centers as well as in the follicular epithelium, which is seen in HT [20]. Furthermore, in a previous study, Harach and Williams [6] characterized plasma cell subsets in Riedel's thyroiditis by immunohistochemistry, demonstrating that IgA plasma cells but not IgG plasma cells were predominant in Riedel's thyroiditis. Therefore, as previously suggested by Li et al. [8, 9], we also considered that Riedel's thyroiditis is unrelated to IgG4-related sclerosing disease.

In conclusion, the present study demonstrated that the majority of cases with IgG4 HT and non-IgG HT showed an interfollicular distribution pattern of IgG4- and/or IgG-positive plasma cells. Moreover, a minority of IgG4 HT (7%) and non-IgG4 HT contained IgG/kappa light chain-restricted germinal centers. However, the number of these cases is too limited to clarify the clinicopathological significance at present.

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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

		Follicles		Interfollicular area							Sinus
	Size (cm)	Foliclular 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.

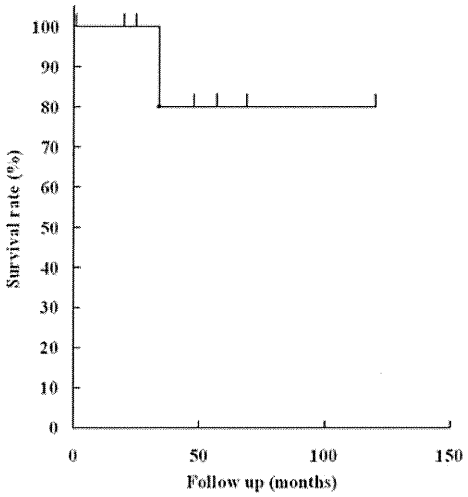

Fig. 1. Overall survival of the 8 patients with atypical lymphoplasmacytic immunoblastic proliferation.

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

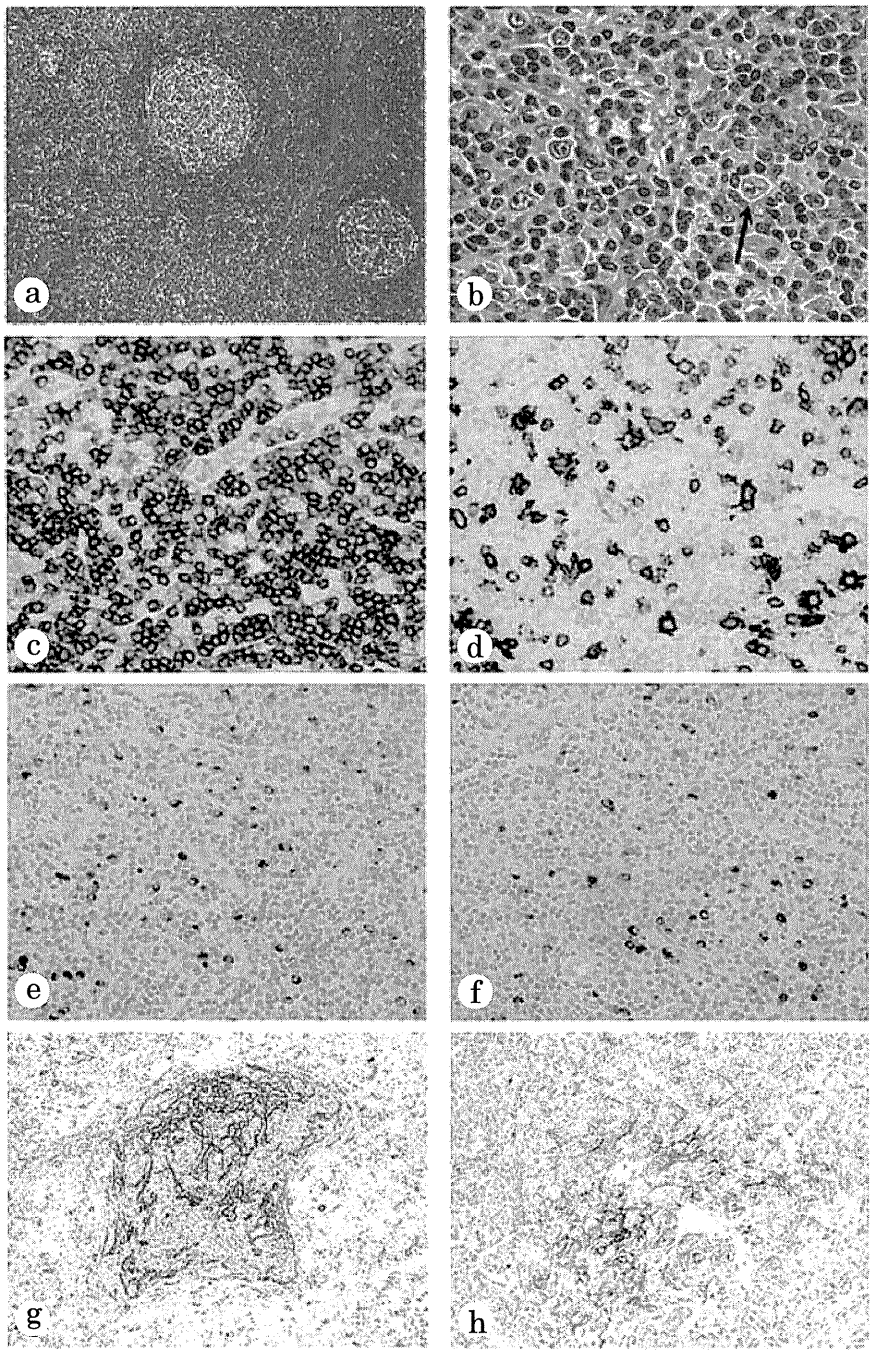

Fig. 2. Histological and immunohistochemical findings of atypical lymphoplasmacytic immunoblastic proliferation. (***2a***) Low-power field of biopsy specimen. The lesion was characterized by diffuse paracortical hyperplasia with small vessel proliferation and a hyperplastic germinal center. Case 7, 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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