PCR assay for IgH gene demonstrated an only germ line bands with IgH chain probes in all eight cases.

Discussion

The HV type of CD accounts for approximately 90% of cases[2-4]. Majority of the CD located in the retroperitoneum were HV type of CD[14, 15]. However, in the present series, four of the eight cases of retroperitoneal CD were PC type of CD. It has been said that majority of the PC type of CD were located in the peripheral lymph node[2, 4]. However, all four case of PC type of CD were extranodal lesions. These clinicopathological findings are quite different from previous description.

Some degree of sclerosis is common in follicular lymphoma (FL), particularly in those involving in retroperitoneum and groin[16-18]. When sclerosis is prominent, the term "sclerosing variant of FL" has previously been applied[18]. Follicular lymphoma rarely shows lymphoid follicles mimicking HV type of CD[16,19]. One of our four cases HV type of CD showed prominent interfollicular sclerosis. However, lymphoid follicles of the present four HV type were CD10-[16, 17]. Moreover, genotypic study demonstrated polytypic nature of the B-lymphocytes.

PC type of CD should be differentiated from low-grade B-cell lymphoma showing prominent plasma cell differentiation particularly marginal zone B-cell lymphoma and multiple myeloma involving extramedullary organs[16,17, 20]. However, immunohistochemical and genotypic study demonstrated polytypic nature of the B-lymphocytes. Moreover, there were no CD43+ CCL-cells and/or CD56+ plasma cells in any of the four lesions[16,17, 20, 21].

Interestingly, immunohistochemical study demonstrated numerous IgG4+ plasma cells accounting for more than 50% of IgG+ cells in three (nos. 3, 5 and 7) of the four lesions[8]. The obstructive phlebitis which is one of the characteristic histological findings of IgG4-related disorders in one case (no. 6).[4-7] The fibrous sclerosis in the interfollicular area were also seen in Case 3[4-8].

Serum IgG4 concentration was increased in two cases (nos. 3 and 7) whereas serum IgG4 concentration was within the normal range in Case 5. However, postoperatively serum IgG4 level may decrease in the normal range in Case 5. These three cases appear IgG4-related disorders.

Yoshizaki et al. demonstrated that abnormal clinical findings such as general fatigue,

anemia and polyclonal hyperganmmaglobulinemia may be related to a high level of IL-6 in the PC type of CD.[22] However, the serum IL-6 level was within the normal range in two cases (nos. 3 and 7). Moreover, there were no clinical characteristics of PC type of CD in any of the four cases.[2, 3, 22, 23].

The remaining one (no.6) showed similar histopathological findings of other three cases including thrombophlebitis and the fibrous sclerosis in the interfollicular area. This case indicated that at least, a portion of PC type of CD is unrelated to the IgG4 related disorder. In other word, PC type of CD contains heterogeneous disease entity.

Good responsiveness to glucocorticoid therapy is seen in IgG4-related disorder.[7], whereas, PC type of CD occasionally resistant to corticosteroid therapy [23]. From a therapeutic perspective, it is important to discriminate IgG4-related disorder from PC type of CD.

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Figure Legends

Fig.1

a. Low power field of the affected lymph node. There were nodules of mantle cells with inconspicuous germinal centers. These nodules were penetrated by small vessels. Note a large nodule of mantle cells contained multiple small atrophic GCs (arrow). HE Case 1 x10

b. High-power field of a GC of Fig.1a. Note the nuclear enlargement of FDCs. HE Case 1x100

c. Medium-power field of the lesion. Note marked small vessel proliferation and sclerosis in the interfollicular area. HE Case 4 x25

d. On low-power field, numerous lymphoid follicles with active germinal centers were present. Note the fibrous sclerosis in the interfollicular area Case 3 HEx10

e. On high power field, numerous infiltrating plasma cells including Mott cells were seen in the interfollicular area. There was neither marked proliferation of blood vessels. Case 3 HE x100

f. Elastica van Gieson stain demonstrated phlebitis in Case 6 x10

Fig. 2

Immunostaining for light chain determinant of the immunoglobulin demonstrated the polytypic nature of mature plasma cells. (a)Kappa (b)Lambda Case 3 x100

c. There were numerous IgG-positive cells in the paracortical area. Case 3 x100

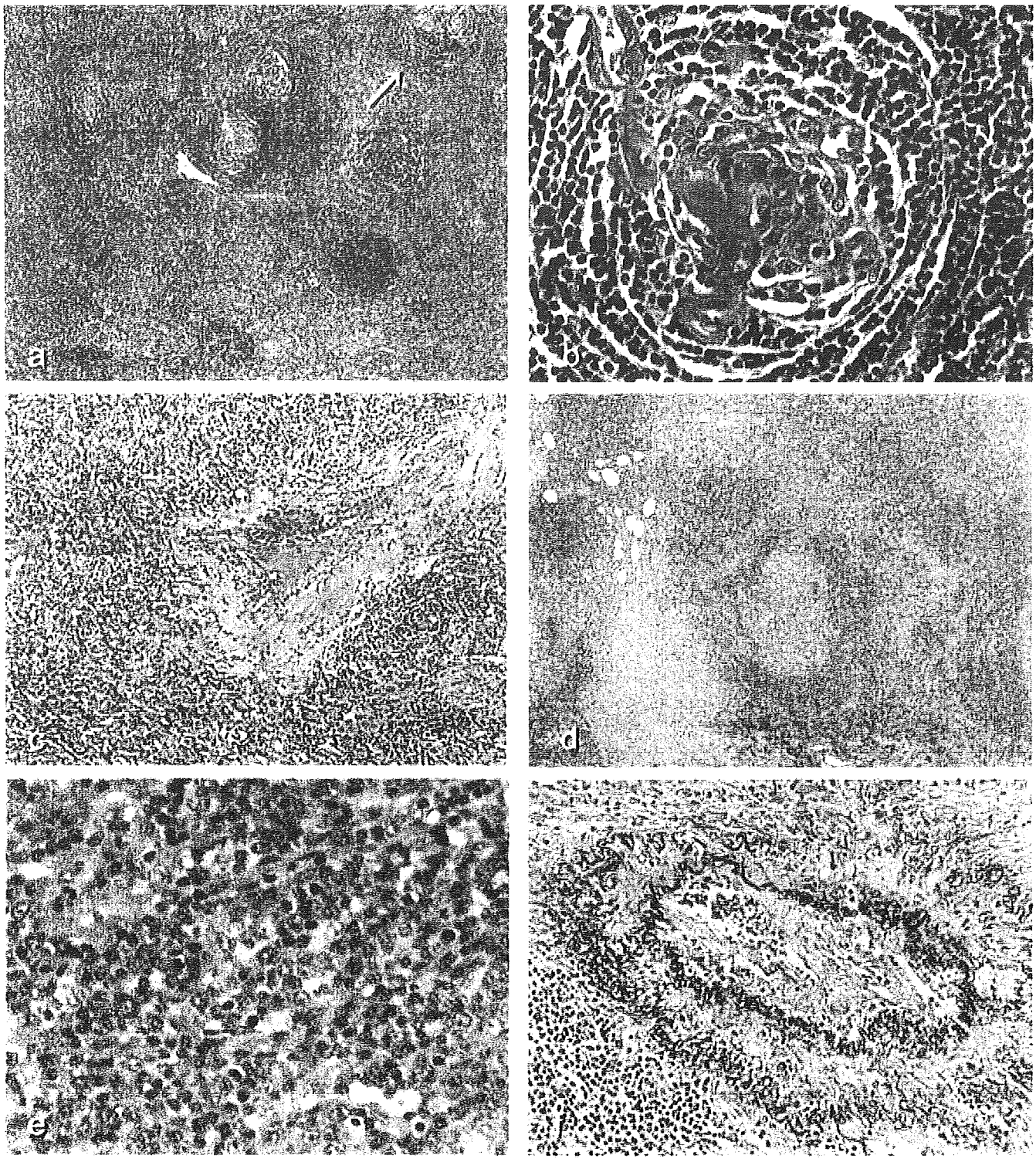
d. Fifty percent of the IgG-positive plasma cells were IgG4-positive. Case 3 x100

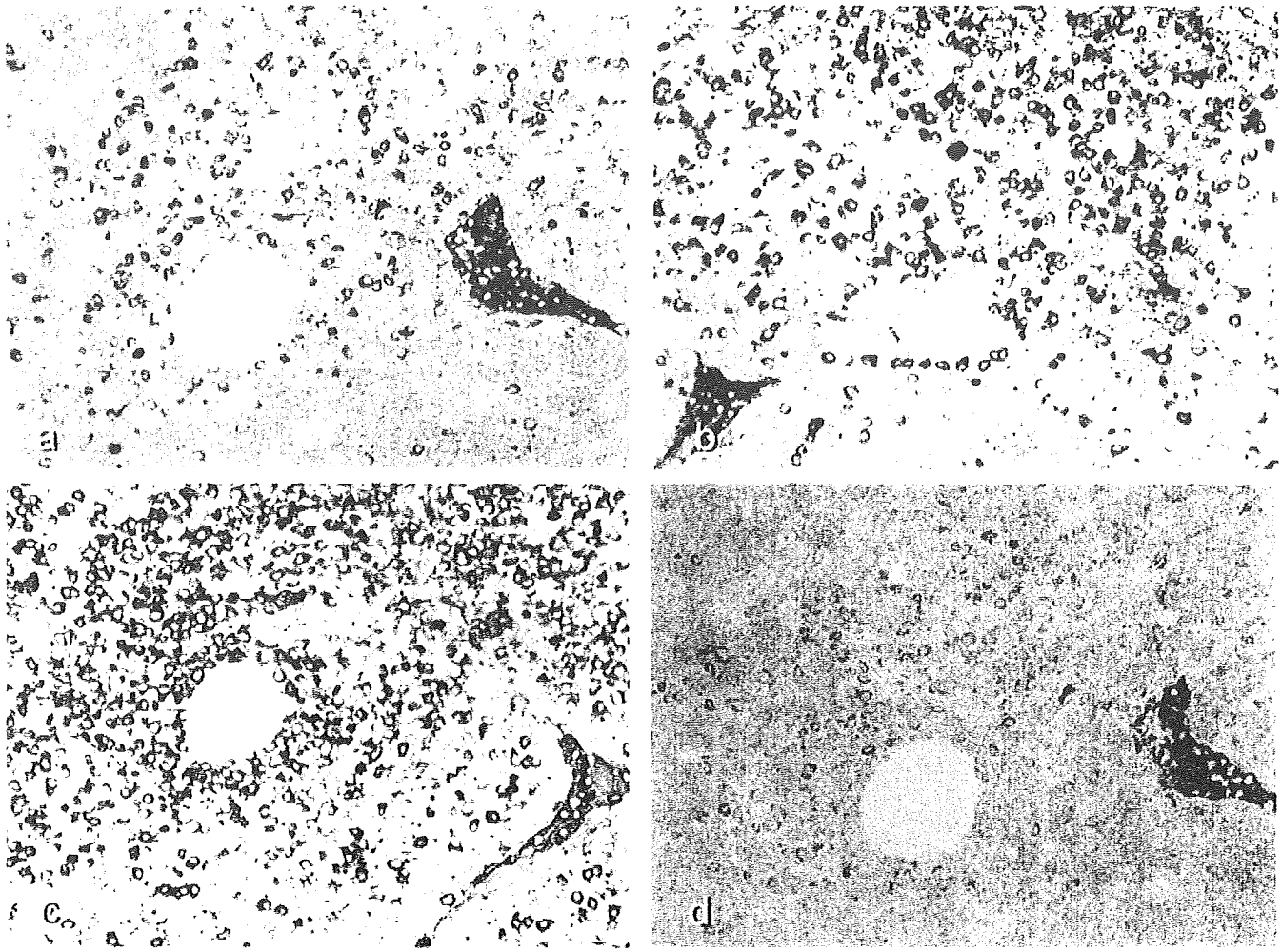
Table. Summary of eight cases

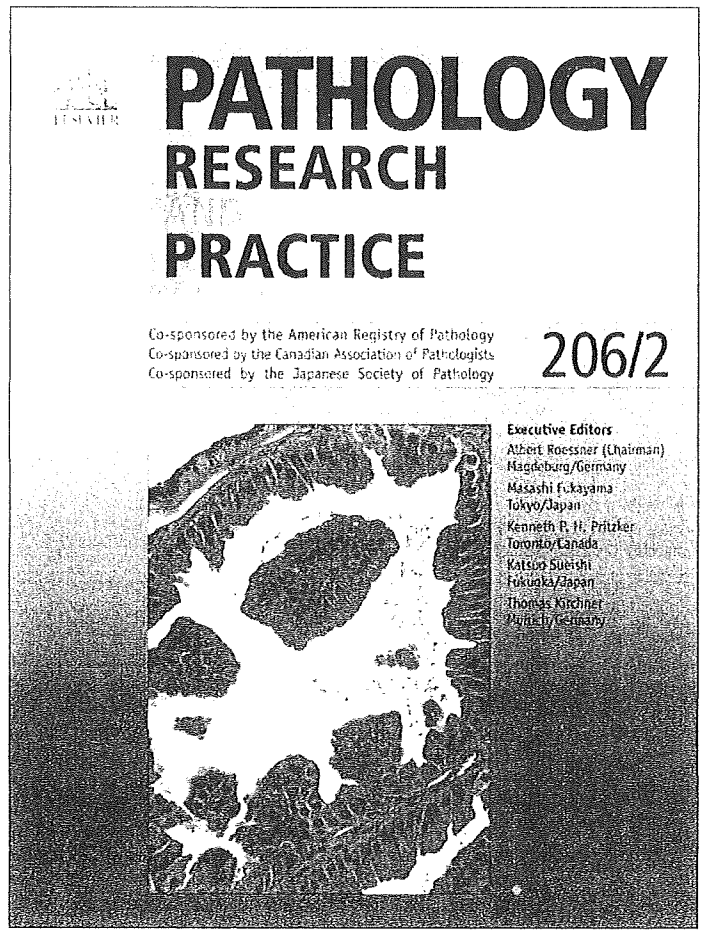
	Age/ Gender	Site of the tumor	Size (cm)	Systemic symptom	IgG4* (mg/dl)	IL-6** pg/ml	Therapy	Outcome	Hist.
1	21/F	Rt. retroperitoneum	5	—	NE	NE	Resection	17m A(—)	HV
2	46/F	Retroperitoneum	5	—	NE	NE	Resection	4m A(—)	HV
3	47/F	Bil. ureter	1.5	—	478	1.6	Biopsy+ Predonisone	6m A(+)	PC
4	59/M	Retroperitoneum	5	—	NE	NE	Resection	Lost	HV
5	68/F	Lt. renal hilum	3	—	101	NE	Resection	11m A(—)	PC
6	71/F	Lt. Ureter	3.5	—	75.9	NE	Resection	12m A(—)	PC
7	73/M	Lt. Ureter	3.5	fever	412	2.33	Resection	22m A(—)	PC
8	75/M	Lt. renal hilum	2	—	NE	NE	Resection	108m A(—)	HV

Abbreviations; IL-6, interleukin-6; Hist., histology; Rt., right; Lt., left; Bil. bilateral; NE, not examine; m, months; A(—), alive without disease; A(+), alive undertreatment; HV, hyaline-vascular; PC, plasma cell

* Normal range; <135mg/dl
** Normal range; <4.62pg/ml







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Original Article

Presence of immunoglobulin heavy chain rearrangement in so-called "plasma cell granuloma of the lung"

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SUMMARY

Inflammatory pseudotumor of the lung appears to be a set of heterogeneous disorders. Histologically, three subtypes of pulmonary IPTs have been delineated. Among these, plasma cell granuloma (PCG) is characterized by prominent lymphoplasmacytic infiltration, and PCG has been added to the list of differential diagnostic problems of mucosa-associated lymphoid tissue (MALT) type lymphoma. To investigate the presence or absence of monoclonal B-cell proliferation, we analyzed the immunohistological and genotypic findings in three cases of pulmonary PCGs. Histologically, the three lesions were characterized by severe infiltration of mature plasma cells, plasmacytoid cells, and small lymphocytes intermixed. Scattered Russell bodies (intracytoplasmic inclusions) were present in all three cases, but there were no Dutcher bodies (intranuclear inclusions) or centrocyte-like cells. Immunohistochemical studies of light chain determinants demonstrated the polytypic nature of B-cells. There was no $CD5^+$, $CD43^+$ or cyclin $D1^+$ B-lymphocytes in any of the three lesions. There were no lymphoepithelial lesions detected within any of the three lesions even by immunostaining for cytokeratin. However, polymerase chain assay for immunoglobulin heavy chain gene demonstrated a clonal band in one of the three cases. It currently remains unclear whether this one case, demonstrating IgH gene rearrangement in our series, could be a sign of the prelymphomatous stage (e.g. incipient MALT type lymphoma) or merely represents an exaggeration of normal B-cell clonal response.

Introduction

So-called "Inflammatory pseudotumors (IPTs)" affect almost all major organs including the lungs [3,20]. Histologically, IPTs are characterized as an irregular proliferation of myofibroblasts intermixed with inflammatory cells, mainly lymphocytes and plasma cells [3,20]. Histologically, IPT of the lung has been classified into three histological types: (i) fibrohistiocytic type, (ii) plasma cell granuloma (PCGs), and (iii) largely sclerosed or fibrosed type [20]. True neoplastic proliferations of mesenchymal or dendritic cells have been reported in some IPTs [2,3,13,21]. Pulmonary IPTs have also been associated with previous viral infections such as human herpes virus type-8 (HHV-8) [8]. Recently, Zen et al. [22] demonstrated that some pulmonary PCGs represent an IgG4-related sclerosing disease. Rarely, pulmonary IPTs have also been associated with B-cell lymphoma [13]. Moreover, PCG has been added to the list of differential diagnostic problems of extramedullary plasmacytoma (EMP) [12]. To examine the presence or absence of monoclonal B-cell proliferation, we analyzed the immunohistological and genotypic findings in three cases of pulmonary PCGs.

Materials and methods

The tissue specimens were fixed in formalin solution, routinely processed and embedded in paraffin. For light microscopic examination, the sections were stained with hematoxylin–eosin (HE) and elastica van Gieson (EVG) stain.

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Immunohistochemical studies were performed using the antigen retrieval method on the avidin–biotin–peroxidase method or Ventana automated (BenchMark™) stainer according to the manufacturer's instructions.

The panel of antibodies included human immunoglobulin light chains (kappa and lambda) (Dako A/S, Glostrup, Denmark), IgG (Novocastra, Newcastle, UK), IgA (Novocastra), IgM (Novocastra), MCO011 (IgG4;Binding Site, Birmingham, UK), PS-1 (CD3; Immunotech, Marseille, France), 4C7 (CD5; Novocastra), L26 (CD20; Dako), a cocktail of 2G9 (CD21; Novocastra) and RB L25 (CD35; Novocastra), 1B12 (CD 23; Novocastra), DFT-1 (CD43; Dako), 1B16 (CD56; Novocastra), PGM-1(CD68; Dako), 5A4 (CD246 [anaplastic lymphoma kinase, ALK]; Novocastra), SP4 (Cyclin D1; Nichirei Co., Tokyo, Japan), AE1/3 (Dako), V9 (vimentin; Dako), D33 (desmin; Dako), HHF35 (muscle-specific actin; Nichirei Co.), S-100 (Dako), and 137B1 (human herpes virus type-8, Novocastra). Sections with known reactivity for 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.

DNA was extracted from the paraffin-embedded section. The variable region (CDR2 and FW3) and VDJ region (CDR3) of the immunoglobulin heavy chain (IgH) gene were amplified by semi-nested PCR, using primers of FR2B, LJH, and VLJH, according to a previously described method [14]. Primers were as follows: 5′-CCGG(A/G)AA(A/G)(A/G) GTCTGGAGTGG-3′, as up-stream consensus V region primer (FR2B); 5′-TGAGGAGACGGTGACC-3′, as a consensus J region primer (LJH); 5′-GTGACCAGGGT [A/C/G/T] CCTTGGCCCCAG-3′, as a consensus J region primer (VLJH). PCR products were estimated to be about 200–300 bps in length.

The API2-MALT1 fusion transcript was examined using formalin-fixed and paraffin-embedded tissue according to the method recently described by us [11].

Results

Clinical findings

The clinical histories of PCG are summarized in Table 1. Only one (no.1) of three cases showed bloody sputum. The remaining two cases (nos. 2 and 3) were asymptomatic but had shown abnormal shadows on chest radiograph during a medical check-up. Chest radiographs and computed tomography demonstrated a solitary nodule in the peripheral pulmonary field in two cases (nos. 1 and 3) and four nodules on the peripheral pulmonary field of the bilateral lobes in the remaining one case (no. 2). One patient (no. 3) had a history of rectal cancer before the episode of PCG. Two cases (nos. 2 and 3) showed mediastinal lymphadenopathy. There was no other evidence of disease in any of the three cases.

Elevated serum IgG level had been recorded in one (no. 2) of the three cases. Antinuclear antibody was detected in Case 2. Serum IgG4 level was within the normal range in of two cases (nos. 1 and 3) examined.

Clinically, two cases (nos. 1 and 2) were suspected of having primary lung cancer and Case 3 was suspected of having metastatic rectal cancer. Two cases (nos. 2 and 3) showed hilar lymphadenopathy. However, lymph node biopsies were not performed.

Pathological findings

Macroscopically, two lesions (nos. 1 and 3) were solitary tan and firm and were relatively well circumscribed without fibrosis (Fig. 1a). The remaining case comprised four nodules in the bilateral lungs.

Histologically, the lesions were characterized by a relatively well-demarcated mass composed of a few lymphoid follicles and chronic inflammatory process intermixed with irregular fibrosis (Fig. 1b). The lesions demonstrated severe infiltration of mature plasma cells, plasmacytoid cells, and small lymphocytes (Fig. 1c). Scattered Russell bodies (intracytoplasmic inclusions) and a few immature plasma cells, large transformed lymphocytes, including immunoblasts and histiocytes, were present in all three cases (Fig. 1c), but there were no Dutcher bodies (intranuclear inclusions), centrocyte-like (CCL) cells, or amyloid deposition. There was no remarkable eosinophilic infiltration in any of the three lesions. Three lesions contained scattered multinucleated giant cells. At the boundaries of the nodules, the inflammatory process extended into the adjacent parenchyma, showing fibrous endings of the alveolar septa with lymphoplasmacytic infiltration (interstitial pneumonia pattern) in all three cases (Fig. 1b). EVG staining demonstrated prominent obliterative phlebitis and arteritis in one case (no. 3) (Fig. 1d). However, there was no necrotic area in Case 3.

Staining for CD20, CD3, and CD5 showed the mixed nature of the small lymphocytes. The majority of large transformed lymphocytes, including immunoblasts, expressed B-cell antigen. Immunohistochemical studies of light chain determinants for plasma cells, plasmacytoid cells, and B-immunoblasts have demonstrated a polyclonal pattern (Figs. 1e and f). There were numerous IgG-positive plasma cells with scattered IgA- or IgM-positive plasma cells. However, IgG4-positive cells comprised only 5–10% of the IgG-positive plasma cells. There were no $CD20^+$, $CD5^+$, $CD43^+$, or cyclin $D1^+$ medium-sized lymphocytes in any of the three lesions. Staining with monoclonal antibody, a cocktail of CD21 and CD35 and CD23 highlighted the meshwork of follicular dendritic cells (FDCs). The FDC networks usually showed a normal/reactive pattern. There were no lymphoepithelial lesions

Table 1
Summary of clinical findings.

	Age/ gender	Symptom	Location (number)	Size (cm)	Hilar LA	Autoantibody	IgG4 (mg/ dl)	Treatment	Outcome
1	58/M	Bloody sputum	Right lower lobe, peripheral (1)	4	–	NE	18.1	Video-associated thoracoscopic surgery	4 m alive (–)
2	68/M	–	Bilateral lobe, peripheral (4)	7	+	ANA	NE	Video-associated thoracoscopic surgery	56m alive (+)
3	72/M	–	Left lower lobe (1)	2.5	+	NE	52	Partial lobectomy	4 m alive (–)

Abbreviations: LA, lymphadenopathy; NE, not examined; ANA, antinuclear antibody; m, months;(—), without disease; (+), with disease.
Normal range of IgG4 < 135 mg/dl (Ref [16]).

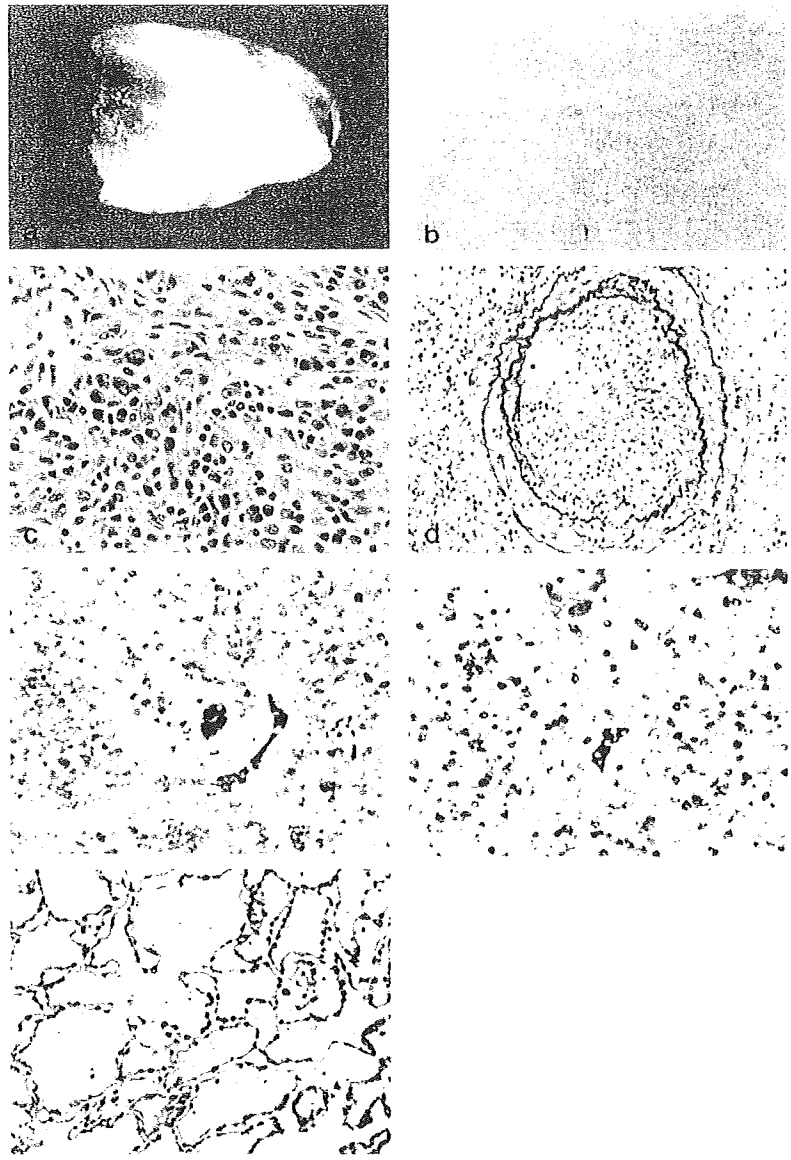

Fig. 1. (a) Cut surface of the resected specimen demonstrated a solitary tan and firm mass that was relatively well circumscribed without fibrosis. Case 3. (b) Low-power field of the lesion. The lesion was a relatively well-demarcated mass composed of a few reactive germinal centers with well-preserved mantle zones and a chronic inflammatory process intermixed with irregular fibrosis. At the boundaries of the nodules, the lesion showed an interstitial pneumonia pattern. Case 3. HE × 10. (c) High-power field of Fig. 1b. The lesion demonstrated severe infiltration of mature plasma cells, plasmacytoid cells, and small lymphocytes. Scattered Russell bodies (intracytoplasmic inclusions) and a few immature plasma cells, large transformed lymphocytes, and histiocytes were present in all three cases. Case 3. HE × 100. (d) EVG staining demonstrated obliterative arteritis. Case 3. × 50. Immunostaining for light chain determinant of the immunoglobulin demonstrated the polytypic nature of mature plasma cells. (e) Kappa and (f) Lambda Case 3. × 50. (g) There were no lymphoepithelial lesions on immunostaining of cytokeratin. Case 1. × 50

(LELs) detected even by immunostaining for cytokeratin in any of the three lesions (Fig. 1g).

There were no CD68+, vimentin+, desmin+, muscle-specific actin+, ALK+, S-100+ spindle cell proliferation cells in any of the three lesions. Moreover, there were no cocktails of CD21 and CD35+, CD23+, EBER+ FDCs in any of the three lesions.

There were no HHV-8 or EBER-positive cells in any of the three cases.

Genotypic findings

A discrete band of amplified IgH gene was found in one case (no. 3) (Fig. 2), respectively. In the remaining two cases (nos. 1 and 2), only germ line bands were detected.

Discussion

IPTs of the lung appear to comprise a set of heterogeneous disease entities [20] that include true neoplastic proliferation of mesenchymal cells, post viral infection state, and autoimmune disease (IgG4-related sclerosing disease) [8,16,21,22]. Histologically, in the present three cases, plasmacytic infiltration was the most conspicuous histological finding compared to myofibroblastic proliferation or collagenous stroma, and all of the present three lesions were diagnosed as PCG [21].

A portion of pulmonary IPTs demonstrated anaplastic lymphoma kinase (ALK) and ALK expression on immunohistochemistry [1,4–6]. At present, these cases are recognized as neoplasms with intermediate biologic potential, namely "inflammatory myofibroblastic tumor" [21]. Immunohistochemical cytoplasmic positivity

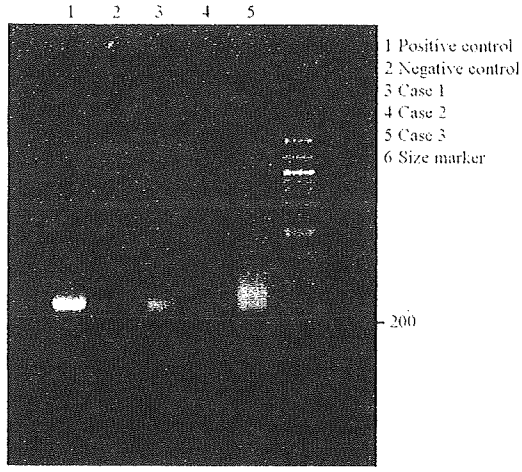

Fig. 2. PCR analysis of three cases for clonal Ig heavy chain rearrangement.

for ALK correlates well with the presence of ALK rearrangements detectable by fluorescent in situ hybridization [4]. However, there were no ALK+ cells in any of the three lesions [21]. Expression of ALK was noted in 40% of the pulmonary inflammatory myofibroblastic tumor [21]. However, there were no CD68+, vimentin+, desmin+, muscle-specific actin+, ALK+, S-100+ spindle cells in any of the three lesions [21]. EBV+ FDC sarcoma appears to be another differential diagnostic problem [3]. However, there were no EBV+ atypical FDCs among the cocktail of CD21 and CD35+ and CD23+FDCs in any of the three lesions [3].

The present three lesions contained numerous IgG+ plasma cells. However, IgG4-positive cells comprised only 5–10% of the IgG-positive plasma cells [16]. Moreover, the serum IgG4-level was within the normal range in two cases examined [16].

Approximately, 70–90% of the primary pulmonary lymphomas are marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type [18]. Various degrees of plasma cell differentiation have been noted in MALT type lymphoma, and plasma cells may occasionally obscure the CCL cells, which are the tumor cells of MALT type lymphoma [18]. Hussong et al. [10] reported five cases of extramedullary plasmacytoma that showed characteristic histological findings of MZBL, including the presence of a marginal zone distribution pattern, CCL cells, reactive lymphoid follicles with or without follicular colonization, and LEL at sites where epithelium was present. Based on these observations, they suggested that a portion of extramedullary plasmacytoma can be regarded as an extreme plasmacytic differentiation of MZBCL [10].

The present three cases should be differentiated from MZBL extreme plasmacytic differentiation. Immunohistochemical studies of light chain determinants demonstrated the polytypic nature of the plasma cells and plasmacytoid cells. There were no CD43+ B-lymphocytes in any of the three lesions [15]. Moreover, there were no LELs detected in any of the three lesions even by immunostaining for cytokeratin. However, PCR assay for IgH gene demonstrated a clonal band in one of the three cases, whereas there was no API2-MALT1 fusion transcript detected in any of the three lesions, although it is detected in approximately 40% of pulmonary MALT type lymphoma [7].

Histologically, vasculitis was observed in numerous arteries and veins in one case (no. 3). Moreover, PCR assay for IgH gene demonstrated a clonal band in Case 3 only. Histological findings of Case 3 were somewhat similar to those of lymphomatoid granulomatosis grade 1 [9,18]. Case 3 should also be differentiated from lymphomatoid granulomatosis grade 1. However, there was no necrotic area in Case 3. Moreover, there were no EBER+ large lymphoid cells in this lesion [9,19].

Recently, Nam-Cha et al. [17] analyzed six florid RFH specimens using immunohistochemistry, IgH-PCR, and microdissected PCR. They found that some germinal centers contained a population of plasma cells and plasmacytoid germinal center cells showing immunoglobulin light chain restriction [17]. In three cases, the monotypic germinal center cells also showed distinct bcl-2 expression [17]. Two cases demonstrated a predominant IgH rearrangement on a florid polyclonal background, and one showed an IgH monoclonal rearrangement on PCR [17]. Nam-Cha et al. [17] reported that only one of the six cases developed follicular lymphoma. As suggested by Nam-Cha et al. in the previous series, it remains unclear whether Case 3, demonstrating IgH gene rearrangement in the present series, could be a sign of prelymphomatous stage (incipient MALT lymphoma) or merely represents an exaggeration of normal B-cell clonal response. However, Case 3 has only undergone a short follow up to date. To clarify this issue, further study is needed.

Acknowledgments

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Progressive transformation of the germinal center of extranodal organs: A clinicopathological, immunohistochemical, and genotypic study of 14 cases

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ABSTRACT

Progressive transformation of germinal center (PTGC) usually affects the peripheral lymph nodes. Little is known about the extranodal PTGC. To clarify the clinicopathological and molecular findings of extranodal PTGC, we studied 14 such cases. Using formalin-fixed, paraffin-embedded sections, we carried out histological and immunohistochemical examinations, as well as in situ hybridization (ISH) and polymerase chain reaction (PCR). Eleven patients were female, and three were male. They were between 44 and 77 years old, with a mean age of 62 years. The large intestine (n=7) was the most frequently involved tissue, followed by skin (n=2) and subcutaneous soft tissue (n=2). Oral cavity, Waldeyer ring, and orbit were affected in one case each. Histologically, 13 cases contained both early stage PTGC and late stage PTGC. The remaining 14th case contained only late stage PTGC. Expansion of the marginal zone was identified in three cases. Immunohistochemical study demonstrated the reactive nature of the B-cells in all 14 lesions. However, PCR study revealed immunoglobulin heavy chain (IgH) gene rearrangement in one of our 14 cases. There was no development of B-cell lymphoma in one lesion with IgH rearrangement. ISH study demonstrated Epstein–Barr virus-encoded small RNA+ cells in three lesions. Compared with PTGC of the peripheral lymph node, PTGC of extranodal sites was characterized by a female predominance, an older age group, and the presence of numerous PTGC at the affected sites. However, the histological findings of extranodal PTG were similar to those of lymph node PTGC. The clinicopathological findings of the extranodal PTGCs appeared to be different from those of lymph node PTGC.

1. Introduction

Progressive transformation of germinal center (PTGC) was initially reported by Lennert and Müller-Hermelink [20], and further studies were subsequently reported by Müller-Hermelink and Lennert [23]. PTGC is larger than the regular germinal center and is composed mainly of mantle zone lymphocytes and remnants of large germinal center cells [1,6,10,20,23,25,27]. The mantle zone is obscured, and the interfollicular areas usually contain small lymphocytes and a few immunoblasts [6,10,23,25,27]. PTGC usually affects peripheral lymph nodes but also rarely occurs at extranodal sites such as the upper aerodigestive tract, including oral cavity and skin [6,9]. Indeed, we have reported cases of PTGC in oral cavity, large intestine, and skin [14–16]. Moreover, we have recently found that PTGC occasionally occurs in localized lymphoid hyperplasia of the large

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intestine (5/16 cases; 30%) [16]. To further clarify the clinicopathological, immunohistochemical, and genotypic findings of extranodal PTGC, we studied 14 such cases.

2. Materials and methods

Sixteen cases were collected from a series treated by one of the authors (M.K.) between January 2000 and August 2009. Eight cases (nos. 2–4, 8, and 10–13) have been reported previously [14–16].

The tissue specimens were fixed in formalin solution, routinely processed, and embedded in paraffin. For light microscopic examination, the sections were stained with hematoxylin–eosin (HE).

A basic immunohistochemical panel, including 4C7 (CD5; Novocastra, New Castle, UK), L26 (CD20; bcl-2 Dako A/S Glostrup, Denmark), DFT-1 (CD43; Novocastra or Dako), SP4 (Cyclin D1; Nichirei Co., Tokyo, Japan), and 124 (bcl-2 Dako A/S Glostrup, Denmark), had been performed in all cases.

When additional slides and/or paraffin blocks were available, immunohistochemical analysis was expanded to include antibodies against human immunoglobulin light chain (kappa and lambda) (Dako or Novocastra), IgD (Dako or Novocastra), IgG (Dako or Novocastra), IgM (Dako or Novocastra), 56C6 (CD10; Novocastra), C3D-1 (CD15; Dako), cocktail of 2G9 (CD21; Novocastra) and RB L25 (CD35; Novocastra), 1B12 (CD23; Novocastra), Ber-H2 (CD30; Dako), NK-1(CD57; Novocastra), and E29 (EMA; Dako). Immunohistochemical studies were performed using the antigen retrieval method on the avidin–biotin–peroxidase method or Ventana-automated (BenchMark^{TM}) stainer according to the manufacturer's instructions.

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

DNA was extracted from paraffin-embedded sections obtained from 11cases (nos. 1, 2, 4–9, 11, 13, and 14). The variable region (CDR2 and FW3) and VDJ region (CDR3) of the immunoglobulin heavy chain (IgH) gene were amplified by semi-nested PCR using primers of FR2B, LJH, and VLJH according to a previously described method [18]. The following primers were used: 5′-CCGG(A/G)AA(A/G)(A/G) GTCTGGAGTGG-3′ as up stream consensus V region primer (FR2B); 5′-TGAGGAGACGGTGACC-3′ as consensus J region primer (LJH); and 5′-GTGACCAGGGT [A/C/G/T] CCTTGGCCCCAG-3′ as consensus J region primer (VLJH). PCR products were estimated to be about 200–300 bps in length.

3. Results

A summary of the 14 cases is given in Table 1.

3.1. Clinical findings

Eleven patients were female and three were male. They were between 44 and 77 years old, with a mean age of 62 years and a median age of 64 years. Large intestine (n=7; nos. 3, 7, 8, 10, and 12–14) was the most frequently involved tissue, followed by skin (n=2; nos. 4 and 11) and subcutaneous soft tissue (n=2; nos. 1 and 6). Oral cavity (no. 2), Waldeyer ring (no. 5), and orbit (no. 9) were affected in one case each. The size of their lesions ranged from 0.3 to 4 cm in diameter (mean=1.1 cm). Eleven patients (nos. 1–3, 5, 6, 8, and 10–14) showed a solitary lesion. One patient each had two cutaneous masses (no. 4) and bilateral orbital tumors (no. 9). The remaining lesion (no. 7) was a confluence of multiple small nodules up to 5 mm in the ileocecal lesions.

None of the patients was diagnosed as having immunodeficiency. One patient each had a history of sleep apnea syndrome (no. 5), chronic thyroiditis (no. 6), and appendectomy (no. 14). One patient (no. 7) was tentatively diagnosed as having marginal zone B-cell lymphoma (MZBL) of mucosa-associated lymphocytic tissue (MALT) type, and the patient received CHOP (cyclophosphamide, doxorubicuin, vincristine, prednisone) therapy. The remaining 14 patients did not receive any medication after tumor resection.

Follow-up information was available for 13 patients (nos. 1–12 and 14) for periods ranging from 2 to 120 months (mean 25 months, median 15 months). One patient (no. 7) developed a recurrent lesion in the same area. None of these 13 patients was affected by malignant lymphoma during the follow-up period.

3.2. Histological findings

Histologically, in a single longitudinal section of the lesion, the PTGC occupied up to 5% of the total follicles in three patients (nos. 1, 6, and 7), 30–50% in three (nos. 2, 3, and 10), and more than 50% in eight (nos. 4, 5, 8, 9, and 11–14).

Table 1
Summary of the clinicopathologic findings of 14 cases.

No.	Age gender	Site of disease	Size (cm)	Follow up	FLy	Ep cl	MZH	Pop cells	EBER	PCR
1	44/F	Subcutaneous	1	3 mA (–)	+	–	–	+	–	P
2	49/F	Oral cavity	1	14 mA (–)	–	+	–	+	+	P
3	50/F	Rectum	0.6	27 mA (–)	+	+	–	+	+[a]	NE
4	51/F	Skin	1	26 mA (–)	+	–	–	–	–	P
5	59/F	Tonsil	4	15 mA (–)	–	–	–	+	–	P
6	61/F	Subcutaneous	1	2 mA (–)	+	–	–	+	–	P
7	64/F	Ileocecal	1	120 mA (+)	+	–	–	–	–	P
8	65/M	Rectum	0.4	40 mA (–)	+	+	–	–	–	P
9	66/M	Bil. orbit	1	4 mA (–)	+	–	–	–	–	M
10	71/F	Rectum	0.5	26 mA (–)	+	+	–	–	+	NE
11	71/M	Skin	1.1	23 mA (–)	+	–	–	–	–	P
12	72/F	Rectum	1	12 mA (–)	+	–	+	–	–	NE
13	73/F	Ascending colon	0.3	Lost	+	+	+	–	–	P
14	77/F	Ileocecal	1	8 mA (–)	+	–	+	–	–	P

Abbreviations; Fly follicular lysis; Epcl, epithelioid cell clusters; MZH, marginal zone hyperplasia; Pop cell; cells resembling popcorn cells; EBER, Epstein-Barr virus - encoded small RNA; PCR, polymerase chain reaction; P, polyclonal; NE, not examined; m, months; A, alive; (–), without disease; (+), with disease.

[a] EBER+ cells located in the epithelial cells as well as PTGC.

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PTGC is characterized by the presence of large nodules of lymphocytes, often three to four times the size of normal reactive germinal centers (Fig. 1a). In PTGC, small lymphocytes migrate into the germinal center in a multifocal fashion, progressively accumulating within, expanding, and then disrupting the germinal centers. At an early stage [10], germinal centers assume unusual shapes or break up without clear demarcation of the germinal center and mantle zone (Fig. 1a). These germinal center cell clusters contain centroblasts and centrocytes. Mitotic figures and tingible body macrophages are usually evident in the germinal center (Fig. 1b). At a late stage [10], PTGC are composed of large nodules with numerous small lymphocytes and rare centroblasts and centrocytes (Fig. 1d). In addition, a few centroblasts and immunoblasts resembling lymphocytic and/or histiocytic Reed-Sternberg cell variants (L&H cells) were identified in five subjects in a late stage of PTGC [2,28] (nos. 1–3, 5, and 6) (Fig. 1e). In 12 subjects (nos. 1, 3, 4, and 6–14), various numbers of germinal centers had undergone follicular lysis.

Both stages of PTGC were observed in varying degrees in 13 cases. However, the remaining one case (no. 5) was only in the late stage of PTGC.

Expansion of the marginal zone (MZ) was identified in three cases (nos. 12–14) (Fig. 2a). At higher magnification, the MZ contained numerous small lymphocytes and monocytoid B-cells (MBCs) with abundant pale or clear cytoplasm, and medium-sized indented or round nuclei with small inconspicuous nucleoli accompanied by scattered large transformed lymphocytes (Fig. 2b). Scattered plasma cells and histiocytes with or without epithelioid cell features were intermingled with lymphoid cells (Fig. 2b). The interfollicular area was composed of small round lymphocytes mixed with a few immunoblasts and isolated histiocytes. Scattered small to medium-sized clusters of

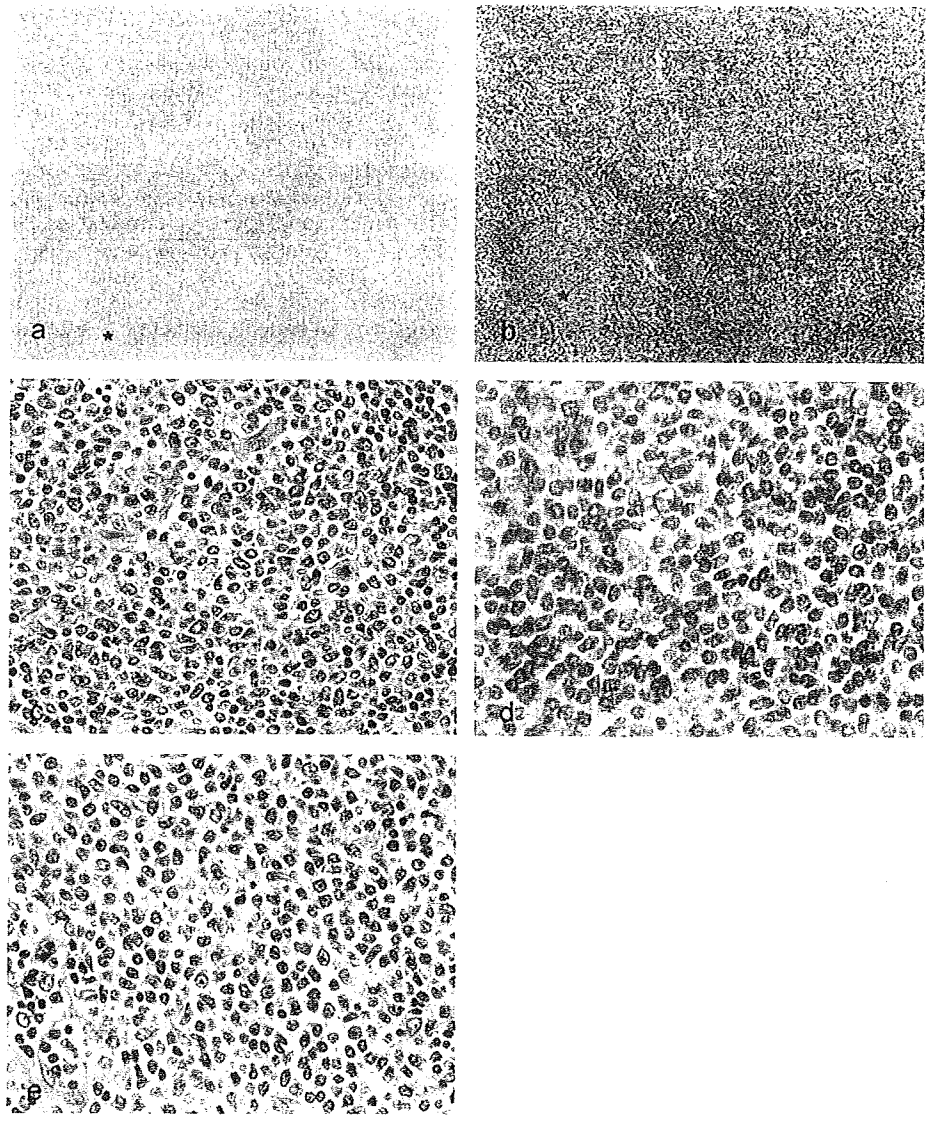

Fig. 1. (a) Low-power field of a lesion showing numerous large PTGC. Note a reactive germinal center(*). Case 5 HE × 10. (b) Early stage PTGC. On low-power field, germinal centers have assumed unusual shapes or break up without clear demarcation of the germinal center and mantle zone. Note expansion of the marginal zone (*) Case 14 HE × 25. (c) High-power field of Fig. 1b. The germinal center cell clusters contained centroblasts and centrocytes. Note a mitotic figure. Case 14 HE × 100. (d) Late stage PTGC. High-power field of Fig. 1a. Note a few centroblasts and immunoblasts resembling L&H cells. Case 5 HE × 150. (e) High-power field of the MZ. The MZ contained numerous small lymphocytes and MBCs with abundant pale or clear cytoplasm, and medium sized indented or round nuclei with small inconspicuous nucleoli accompanied by scattered large transformed lymphocytes. Case 12 HE × 100.

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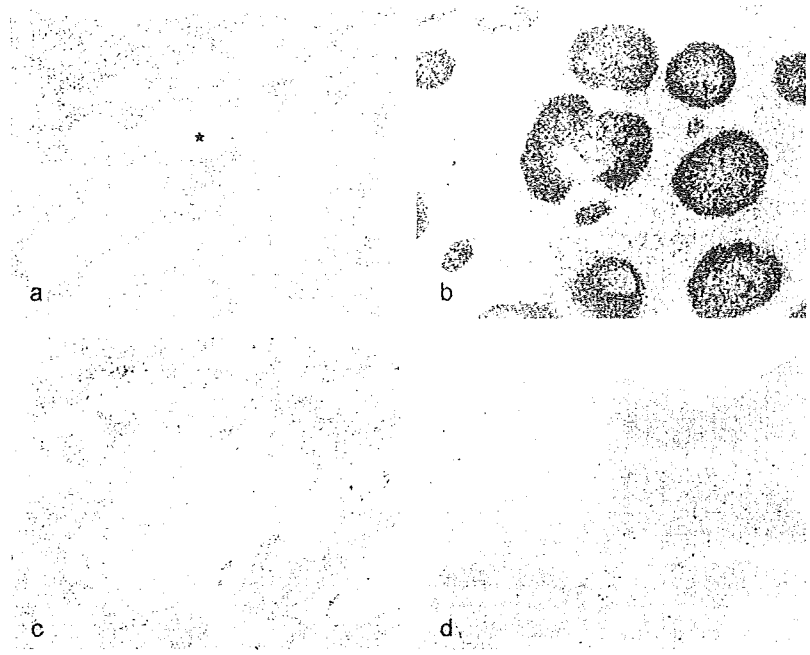

Fig. 2. (a) The germinal center B-cells and MZ B-cells (*) were bcl-2- Case 14 × 10. (b) Small lymphocytes of the PTGC were surface IgD+, but germinal center B-cells were IgD-(*) Case 5 × 10. (c) CD23 immunostain highlighted the meshwork of the FDCs. The FDC meshwork showed complete disruption into clusters in PTGC. Case 14 × 10. (d) ISH studies demonstrated scattered EBER-positive medium- and large- lymphoid cells and crypt epithelium. Case 3 × 10.

epithelioid cells were found in five patients (nos. 2, 3, 8, 10, and 13), while a moderate number of eosinophils were identified in the lesion in one subject (no. 4). Small vessels were not prominent except one case (no. 6). There was prominent interfollicular sclerosis in two cases (nos. 6 and 10).

3.3. *Immunohistochemical findings*

The results of the immunohistochemical examination of the patients enrolled in this study were similar to those of previous reports [1,10,12,25]. Briefly, we found that lymphoid follicles, both typical and PTGC, express CD20 antigen. Germinal center B-cells were CD10+ and CD20+, and were bcl-2-(Fig. 2a), IgM+, and IgD− (Fig. 2b). Immunohistochemical study of the intracytoplasmic light-chain determinant for germinal center cells revealed a polytypic nature.

The infiltrating small lymphocytes are a mixture of mantle zone B-cells and T-cells. Non-neoplastic follicular mantle cells of the PTGC were surface IgD+ (Fig. 2b) and IgM+, CD5−, CD43−, bcl-2+, and CyclinD1−. L&H-like cells were CD30− and EMA−. There were no CD57+ T-cell rosettes identified around the L&H-like cells. Scattered B immunoblasts in the PTGC and interfollicular area expressed CD30 antigen, but not CD15 or EMA. Staining with a cocktail of CD21 and CD35, or CD23 highlighted the meshwork of the follicular dendritic cells (FDCs). The FDC meshwork showed complete disruption into clusters in PTGC (Fig. 2c) [1,10,12,25].

MZ B-cells, including MBCs and large transformed lymphocytes, were CD20+, sIgD−, CD5−, CD10−, CD23−, CD43−, bcl-2− (Fig. 2a), and cyclin D1−. A portion of MBCs were surface IgM+. MZ B-cells, including MBCs and large transformed B-cells, and plasma cells had polytypic intracytoplasmic immunoglobulin light chains.

Destructive lymphoepithelial lesions were not detected even by immunostaining for EMA in any of the 10 cases (nos. 3, 4, 5, 7, 8, 10–14) containing mucosal epithelium in the lesion.

In 13 cases, IgG4 immunostain was performed. IgG4-positive cells comprised up to 30% of IgG-positive plasma cells in one case (no. 9), whereas the remaining 12 cases examined (no. 1–8, 10, 12–14) contained only a few IgG4-postitve plasma cells.

3.4. *EBV findings*

ISH studies demonstrated scattered EBER-positive medium- and large- lymphoid cells and crypt epithelium in three lesions (nos. 2, 3 and 10) (Fig. 2d).

3.5. *Genotypic findings*

PCR analyses for IgH gene demonstrated a polyclonal pattern in10 cases (nos. 1, 2, 4–8, 11, 13 and 14). However, one lesion (no. 9) showed a clonal band on PCR assay for IgH gene (Fig. 3).

4. Discussion

To clarify the clinicopathological features of PTGC in peripheral lymph nodes in Japanese patients, we have previously reviewed 42 cases [13]. PTGC affected males more often than females (male to female ratio, 3:1). Elderly patients accounted for only approximately 30% of the cases. Histologically, less than 10% of cases had PTGC >20% of follicles. Meanwhile, in the present study, PTGCs at extranodal sites were characterized by a female predominance (male to female ratio 1:3), an older age group (elderly patients comprised 60% of cases), and the presence of numerous PTGCs at the affected site. Moreover, it appears that among extranodal sites, PTGS more frequently affected the large

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