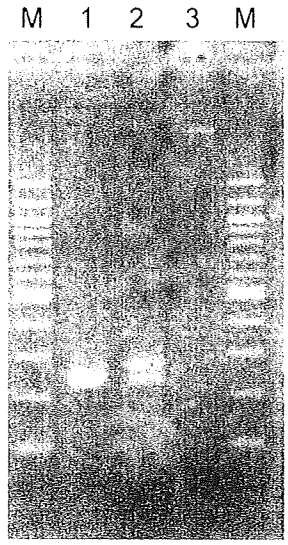

Fig. 3. PCR analysis of Case 9 for clonal Ig heavy chain rearrangement. The lanes contain molecular-weight markers (M), positive control (1), patient DNA (2) and negative control (3).

intestine. However, the histological findings of extranodal PTGS are similar to those of lymph node PTGC [6,10,12,23,27]. The clinicopathological findings of the extranodal PTGCs appear to be different from those of lymph node PTGC.

Immunohistochemical study revealed the reactive nature of the B-cells in all 14 lesions. However, one (no. 9) of our 11 cases examined by PCR demonstrated IgH gene rearrangement. The histological findings of this case (no. 9) were similar to those of other 13 cases. We have recently found such cases with prominent reactive follicular hyperplasia in lymph nodes and large intestine [16,17]. As indicated by Nam-Cha et al., it remains unclear whether this single case is clinically relevant, a sign of prelymphomatous stage, or merely an exaggeration of normal B-cell clonal response in the germinal centers [24]. However, our single case had a relatively short follow-up period. Further study is needed to clarify this issue.

Follicular lymphoma can occur at almost any extranodal site, including orbit, oral cavity, tonsil, large intestine, and skin [4,7,22,30,31]. In particularly the early stage of PTGC should be differentiated from the "floral variant "of follicular lymphoma [5]. Bcl-2 immunostain suggested the reactive nature of lymphoid follicles in our lesion [32]. In follicular lymphoma, the expression of CD10 was seen in both follicular and interfollicular areas [3]. However, in all 14 lesions, CD10 was expressed almost exclusively by follicular center cells, which are characteristic findings of reactive follicular hyperplasia. Immunohistochemical studies of intracytoplasmic light-chain determinant for germinal center cells disclosed a polytypic nature.

Mantle cell lymphoma appears to be the most important differential diagnostic problem because the pseudonodular growth pattern of mantle cell lymphoma resembles the histological findings of late stage PTGC [33,35]. Indeed, one (no. 5) of our 14 cases contained numerous late stage PTGC. The tumor cells of mantle cell lymphoma are small lymphoid cells with slightly indented nuclei and scant cytoplasm [33,35]. However, immunohistochemical study demonstrated that proliferating cells were surface IgD+ and IgM+, CD5 – CD43 –, Cyclin D1 –, suggesting a non-neoplastic mantle cell nature [35].

MZ hyperplasia was present in three patients (21%) of the present series. Tumor cells of extranodal mucosa-associated lymphoid tissue (MALT) type are small- to-medium lymphocytes showing a moderate amount of clear cytoplasm, indented or round nuclei, and absent or small nucleoli (centrocyte-like cells) [11]. Centrocyte-like cells are similar cytological findings of the reactive MZ B-cells. However, there were no CD43+ and/or bcl-2+ MZ B-cells in any of the three lesions demonstrating MZ hyperplasia [19,32]. Moreover, immunophenotypic study demonstrated the polytypic nature of the intracytoplasmic immunoglobulins MZ B-cells.

Very rarely, nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) can show extranodal localization, including Waldeyer's ring, skin, soft tissue, and large intestine [2,28]. PTGC should be differentiated from NLPHL. One of the characteristic immunohistological findings of NLPHL is the presence of CD57+ lymphocyte rosettes around the L&H Reed-Sternberg cell variants. However, there were no CD57+ cells rosettes around the L&H-like cells in any of our cases [28]. Moreover, L&H Reed-Sternberg cell variants are occasionally EMA+. However, there were no EMA+ L&H-like cells in any of our cases [28].

As suggested by Hansmann, PTGC is thought to result from antigen stimulation during particular immunological conditions [6]. However, the etiology of extranodal PTGC is unclear. Weiss et al. have previously demonstrated EBER-positive lymphocytes in the majority of PTGC cases [34]. We also found scattered EBER+ cells in three (rectum=2, oral cavity=1) of the 15 cases. EBV may be involved in the etiology of a subset of PTGC at extranodal sites. However, reactivity of lymphoid cells for EBV has been reported in lymphoid tissues obtained from a high percentage of "normal" individuals [8]. Further study is needed to clarify this issue.

Sato et al. have recently reported lymph node lesions of IgG4-related disorders occasionally showing PTGC [29]. However, IgG4+ plasma cells accounted for up to 30% of IgG-positive plasma cells in only one case [21]. At least, it appears that the majority of extranodal PTGS are not associated with IgG4-related disorders.

Uncited reference

Q1

[26].

Acknowledgments

This work was supported by Intractable Diseases, the Health and Labour Sciences Research Grants from the Ministry of Health, Labor, and Welfare (H21-112)

References

[1] C.C. Chang, V. Osiupov, S. Wheaton, S. Tripp, S.L. Perkins, Follicular hyperplasia, follicularlysis, and progressive transformation of germinal centers. A sequential spectrum of morphologic evolution in lymphoid hyperplasia, Am. J. Clin. Pathol. 120 (2003) 322–326.
[2] K.L. Chang, O.W. Kamel, D.A. Arber, D. Horyd, L.M. Weiss, Pathologic features of nodular lymphocyte predominance Hodgkin's disease in extranodal sites, Am. J. Surg. Pathol. 19 (1995) 1323–1324.
[3] A. Dogan, E. Bagdi, P. Munson, P.G. Isaacson, CD10 and BCL-6 expression in paraffin sections of normal lymphoid tissue and B-cell lymphomas, Am. J. Surg. Pathol. 24 (2000) 846–852.
[4] J.A. Ferry, C.Y. Fung, N. Zukerburg, M.J. Lucarelli, R.P. Hasserjian, F.I. Preffer, N.L. Harris, Lymphoma of the ocular adnexa: a study of 353 cases, Am. J. Surg. Pathol. 31 (2007) 170–184.
[5] J.J. Goates, O.W. Kamel, D.P. LeBrun, D. Benharroch, R.F. Dorfman, Floral variant of follicular lymphoma. Immunological and molecular studies support a neoplastic process, Am. J. Surg. Pathol. 18 (1994) 37–47.
[6] M.L. Hansmann, C. Fellbaum, P.K. Hui, P. Moubayed, Progressive transformation of germinal centers with an without association to Hodgkin's disease, Am. J. Clin. Pathol. 93 (1990) 219–226.
[7] N.L. Harris, S.H. Swerdlow, E.S. Jaffe, G. Ott, B.N. Nathwani, D. de Jong, T. Yoshino, D. Spagnolo, Follicular lymphoma, in: S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, V. Thiele, J.W. Vardiman (Eds.), WHO Classification of Tumours of Haematopoietic and Lymphoid Tssues, IARC-press, Lyon, 2008, pp. 220–226.

Please cite this article as: M. Kojima, et al., Progressive transformation of the germinal center of extranodal organs: A clinicopathological, immunohistochemical, and genotypic study of 14 cases, Pathol. Res. Practice (2010), doi:10.1016/j.prp.2009.12.001

[8] S.D. Hudnall, Y. Ge, L. Wei, N.-P. Yang, H.-Q. Wang, T. Chen, Distribution and phenotype of Epstein-Barr virus-infected cels in human pharyngeal tonsils, Mod. Pathol. 18 (2005) 519–527.
[9] E. Hyjek, G. Kelènyi, Pseudolymphomas of the stomach: a lesion characterized by progressively transformed germinal centers, Histopathology 6 (1982) 61–68.
[10] H.L. Ioachim, L.J. Medeiros, Ioachim's Lymph Node Pathology, 4th ed, Lippincott, Williams & Wilkins, Philadelphia, 2009, pp. 186–189.
[11] P.G. Isaacson, J. Spencer, Malignant lymphoma of mucosa-associated lymphoid tissue, Histopathology 11 (1987) 445–462.
[12] D. Jones, Dismantling the germinal center: comparing the process of transformation, and fragmentation of the lymphoid follicles, Adv. Anat. Pathol. 9 (2002) 129–138.
[13] M. Kojima, S. Nakamura, T. Motoori, H. Itoh, K. Shimizu, N. Yamane, Y. Ohno, S. Ban, K. Yoshida, K. Hoshi, T. Oyama, S. Shimano, S. Sugihara, N. Sakata, N. Masawa, Progressive transformation of germinal centers. A clinicopathological study of 42 Japanese patients, Int. J. Surg. Pathol. 11 (2003) 101–107.
[14] M. Kojima, S. Nakamura, M. Iijima, T. Yoshizumi, N. Sakata, N. Masawa, Follicular lymphoid hyperplasia of the oral cavity representing progressive transformation of germinal center. A case report, APMIS 113 (2005) 221–224.
[15] M. Kojima, S. Sakurai, K. Shimizu, H. Itoh, B-cell cutaneous lymphoid hyperplasia representing progressive transformation of germinal center, A report of two cases. Int. J. Pathol. 2008 as doi:10.1177/1066896908323097.
[16] M. Kojima, N. Nakamura, H. Itoh, T. Motoori, K. Hoshi, Y. Enomoto, T. Johshita, H. Nakamine, Histological variety of localized lymphoid hyperplasia of the large intestine: histopthological, immunohistochemical and genotypic findings of 16 cases, JCEH 49 (2009) 15–22.
[17] M. Kojima, N. Nakamura, K. Murayama, T. Igarashi, M. Matsumoto, H. Matsuda, N. Masawa, I. Miura, Y. Morita, Reactive lymphoid hyperplasia with giant follicles associated with a posttherapeutic state of hematological malignancies. A report of eight cases. Tumori 100 (2010) (in press).
[18] T. Kuze, N. Nakamura, Y. Hashimoto, M. Abe M, Most of CD30+ anaplastic large cell lymphoma of B cell type show a somatic mutation in the IgH V region genes, Leukemia 12 (1998) 753–757.
[19] R. Lai, L.M. Weiss, K.L. Chang, D.A. Arber, Frequency of CD43 expression in non-Hodgkin lymphoma. A survey of 742 cases and further characterization of rare CD43+ follicular lymphomas, Am. J. Clin. Pathol. 111 (1999) 488–494.
[20] K. Lennert, H.K. Müller-Hermelink, Lymphocyten und ihre Funktionsformen Morphologie. Organization und immunologische Bedeutung, Verh. Anat. Ges. 69 (1975) 19–62.
[21] Y. Masaki, L. Dong, N. Kurose, K. Kitagawa, Y. Morikawa, M. Yamamoto, H. Takahashi, Y. Shinomura, K. Imai, T. Saeki, A. Azumi, S. Nakada, E. Sugiyama, S. Matsui, T. Origuchi, S. Nishiyama, I. Nishimori, T. Nojima, K. Yamada, M. Kawano, Y. Zen, M. Kaneko, K. Miyazaki, K. Tsubota, K. Eguchi, K. Tomoda, T. Sawaki, T. Kawanami, M. Tanaka, T. Fukushima, S. Sugai, H. Umemura, et al., Proposal for a new clinical entity. IgG4-positive multi-organ lymphoproliferative syndrome:analysis of 64 cases of IgG4-related disorders, Ann. Rheum. Dis. 68 (2009) 1310–1315.
[22] J. Menárguez, M. Mollejo, R. Carrión, H. Oliva, C. Bellas, J. Forteza, C. Martin, C. Ruiz-Marcellán, M. Morente, V. Romagosa, R. Cuena, M.A. Piris, Waldeyer ring lymphomas. A clinicopathological study of 79 cases, Histopathology 24 (1994) 13–22.
[23] H.K. Müller-Hermelink, K. Lennert, The cytologic, histologic, and functional bases for a modern classification of lymphomas, in: K. Lennert, N. Mohri, H. Stein, E. Kaiserling, H.K. Müller-Hermelink (Eds.), Malignant Lymphomas other than Hodgkin's Disease, Springer, Berlin, 1978, pp. 1–71.
[24] S.H. Nam-Cha, B. San-Millán, M. Mollejo, M. García-Cosio, G. Garijo, M. Gomez, R.A. Warnke, E.S. Jaffe, M.A. Piris MA, Light-chain restricted germinal centers in reactive lymphadenitis: report of eight cases, Histopathology 52 (2008) 436–444.
[25] P.L. Nguyen, J.A. Ferry, N.L. Harris, Progressive transformation of germinal centers and nodular lymphocyte predominance Hodgkin's disease. A comparative immunohistochemical study, Am. J. Surg. Pathol. 23 (1999) 27–33.
[26] N. Pimpinell, E. Berti, G. Burg, L. Ducan, N.L. Harris, E.S. Jaffe, H. Kerl, M. Kurrer, W. Kempf, C.L.J.M. Meijer, M. Santucci, S.H. Swerdlow, R. Willemze, Cutaneous follicle centre lymphoma, in: P.E. LeBoit, G. Burg, D. Weedon, A. Sarasin (Eds.), WHO Classification of Tumours of Pathology and Genetics of Skin Tumours, IARCpress, Lyon, 2006, pp. 196–197.
[27] S. Poppema, E. Kaiserling, K. Lennert, Nodular paragranuloma and progressive transformed germinal center. Ultrastrural and immunohistologic findings, Virchows Arch. [B] 31 (1979) 211–225.
[28] S. Poppema, G. Delsol, S. Pileri, H. Stein, S.H. Swerdlow, R.A. Warnke, E.S. Jaffe, Nodular lymphocyte predominant Hodgkin lymphoma, in: S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, V. Thiele, J.W. Vardiman (Eds.), WHO Classification of Tumours of Haematopoietic and Lymphoid Tssues, IARCpress, Lyon, 2008, pp. 323–325.
[29] Y. Sato, M. Kojima, K. Tanaka, T. Morito, H. Asaoku, T. Takeuchi, K. Mizobuchi, M. Fujihara, K. Kuraoka, T. Nakai, K. Ichimura, T. Tanaka, M. Tamura, Y. Nishikawa, T. Yoshino, Systemic IgG4- related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease, Mod. Pathol. 22 (2009) 589–599.
[30] J. Shia, J. Teruya-Fledstein, D. Pan, A. Hegde, D.S. Klimstra, R.S.K. Chaganiti, J. Qin, C.S. Portlock, D.A. Filipa, Primary follicular lymphoma of the gastrointestinal tract. A clinical and pathologic study of 26 cases, Am. J. Surg. Pathol. 26 (2002) 216–224.
[31] C.C. Solomides, A.S. Miller, R.A. Christman, J. Talwar, H. Simpkins, Lymphomas of the oral cavity: histology, immunologic type, and incidence of Epstein-Barr virus infection, Hum. Pathol. 33 (2002) 153–157.
[32] T. Wang, J. Lasota, C.A. Hanau, M. Miettinen, Bcl-2 oncoprotein is widespread in lymphoid tissue and lymphoma but its differential expression in benign versus malignant follicles and monocytoid B-cell proliferation is of diagnostic value, APMIS 103 (1995) 655–662.
[33] D.D. Weiseburger, M.J. Duggan, D.A. Perry, W.A. Sanger, J.O. Armitage, Non-Hodgkin's lymphomas of mantle zone origine, Pathol. Ann. 26 (I) (1991) 139–158.
[34] L.M. Weiss, Y.-Y. Chen, X.-F. Liu, D. Shibata, Epstein-Barr virus and Hodgkin's disease. A correlative in situ hybridizationand polymerase chain reaction study, Am. J. Pathol. 139 (1991) 1259–1265.
[35] Y. Yatabe, R. Suzuki, Y. Matsuno, K. Tobinai, R. Ichinohasama, J.-I. Tamaru, Y. Mizoguchi, Y. Hashimoto, M. Yamaguchi, M. Kojima, N. Uike, M. Okamoto, K. Isoda, K. Ichimura, Y. Morishima, M. Seto, T. Suchi, S. Nakamura, Morphological spectrum of cyclin D-1 positive mantle cell lymphoma: study of 168 cases, Pathology International 51 (2001) 747–761.

Please cite this article as: M. Kojima, et al., Progressive transformation of the germinal center of extranodal organs: A clinicopathological, immunohistochemical, and genotypic study of 14 cases, Pathol. Res. Practice (2010), doi:10.1016/j.prp.2009.12.001

AUTHOR QUERY FORM

	**Journal: PRP** **Article Number: 50599**	**Please e-mail or fax your responses and any corrections to:** **E-mail: corrections.esil@elsevier.macipd.com** **Fax: +44 1392 285878**

Dear Author,

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Articles in Special Issues: Please ensure that the words 'this issue' are added (in the list and text) to any references to other articles in this Special Issue

Uncited references: References that occur in the reference list but not in the text – please position each reference in the text or delete it from the list.
Missing references: References listed below were noted in the text but are missing from the reference list – please make the list complete or remove the references from the text.

Location in article	Query/remark Please insert your reply or correction at the corresponding line in the proof
Q1	Uncited references: this section comprises references that occur in the reference list but not in the body of the text. Please position each reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section. Thank you.

Electronic file usage

Sometimes we are unable to process the electronic file of your article and/or artwork. If this is the case, we have proceeded by:

☐ Scanning (parts of) your article ☐ Rekeying (parts of) your article ☐ Scanning the artwork

Thank you for your assistance.

Pathology – Research and Practice ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.de/prp

Short Communication

Infectious mononucleosis lymphoadenitis showing histologic findings indistinguishable from toxoplasma lymphadenitis. A report of three cases

Masaru Kojima [a,*], Makoto Kashimura [b], Hideaki Itoh [c], Masahiro Noro [d], Hazuki Matsuda [a], Norihumi Tsukamoto [e], Bunshiro Akikusa [d], Nobuhide Masawa [a], Yukio Morita [f]

[a] Department of Anatomic and Diagnostic Pathology, Dokkyo Medical University School of Medicine, Mibu, Tochigi 321-0293, Japan
[b] Department of Hematology, Matsudo City Hospital, Matsudo, Japan
[c] Department of Pathology and Clinical Laboratories, Maebashi Red Cross Hospital, Maebashi, Japan
[d] Department of Pathology, Matsudo City Hospital, Matsudo, Japan
[e] Department of Medicine and Clinical Science, Gunma University School of Medicine, Maebashi, Japan
[f] Laboratory of Food Hygiene, Tokyo Kasei University, College of Nutritional Science, Tokyo, Japan

ARTICLE INFO

Article history:
Received 21 October 2009
Received in revised form
22 December 2009
Accepted 24 December 2009

Keywords:
Infectious mononucleosis
Lymph node/toxoplasmic lymphadenitis
Clinical findings
Polymerase chain reaction

ABSTRACT

Lymph node lesions in infectious mononucleosis (IM) show a marked histologic diversity. We report here three cases of IM lymphadenitis with histologic findings indistinguishable from those of toxoplasmic lymphadenitis. The histologic findings of the three cases presented here showed a histologic triad of toxoplasmic lymphadenitis, including (i) numerous lymphoid follicles with hyperplastic germinal centers; (ii) small clusters or single epithelioid histiocytes; and (iii) multiple foci of monocytoid B-cells. Moreover, all three lesions contained isolated or small clusters of epithelioid histiocytes within the hyperplastic germinal centers and the periphery of lymphoid follicles, which are the most specific histologic findings of toxoplasmic lymphadenitis. However, serologic findings confirmed EBV infection in all three cases. On in situ hybridization, numerous Epstein-Barr virus (EBV)-encoded small RNA (EBER)-positive cells were demonstrated in the germinal center, as well as in interfolicular areas in all three cases. *Toxoplasmosis gondii* infection was excluded in at least one case, based on serologic findings. Polymerase chain reaction analysis also demonstrated that there was no *T. gondii* DNA in the remaining two cases. Two of our three cases showed atypical clinical presentations, including an absence of atypical lymphocytosis in peripheral blood in two cases, age more than 30 years, and an absence of systemic symptoms in one case. It appears that previous descriptions emphasize the differential diagnostic problems between IM lymphadenitis and malignant lymphomas. However, from a therapeutic perspective, it is important to discriminate IM lymphadenitis from toxoplasmic lymphadenitis particularly in patients showing atypical clinical features.

Introduction

Toxoplasmosis is a well-recognized specific cause of a benign lymph node lesion. In this disease, a triad of histologic findings is highly suggestive: reactive follicular hyperplasia, increased monocytoid B-cells (MBCs) in the sinuses, and the presence of clusters or single epithelioid histiocytes that encroach upon and infiltrate the germinal centers [8,15].

Lymph node lesions in infectious mononucleosis (IM) show a marked histologic diversity [2,4,7,17–19]. However, the major changes are reactive hyperplasia of the lymphoid follicles, expansion and distortion of the paracortex, and the dilatation of the sinuses [8,19]. It has been suggested that IM lymphadenitis shows histologic findings similar to those of toxoplasmic lymphadenitis [4,7,19]. However, a detailed histomorphologic description has not yet been given in the literature. We report here three cases of IM lymphadenitis showing indistinguishable histologic findings of toxoplasmic lymphadenitis.

Case report

Case 1

A 21-year-old Japanese woman presented with bilateral neck lymphadenopathy. The patient had noted a sudden onset of non-tender swelling, accompanied by an episode of high-grade fever and sore throat. Physical examination revealed hepatosplenomegaly. Abdominal ultrasonography demonstrated mild hepatomegaly and retroperitoneal lymphadenopathy. Laboratory studies

* Corresponding author. Tel.: +81 28 286 1111x2178; fax: +81 28 286 5171.
E-mail address: mkojima@dokkyomed.ac.jp (M. Kojima).

0344-0338/$ - see front matter © 2010 Published by Elsevier GmbH.
doi:10.1016/j.prp.2009.12.010

demonstrated a white blood cell count of 4800/mm^3 with 23% atypical lymphocytes in peripheral blood. A moderately abnormal liver function was also noted. A left cervical lymph node biopsy was performed. Subsequent serologic tests for Epstein-Barr virus (EBV) showed a positive heterophil agglutination test for specific EBV serology. Serologic tests for *Toxoplasma gondii* demonstrated that a titer of *Toxoplasma* IgM was < 10 ($< \times 10$).

Case 2

A 22-year-old Japanese man presented with right neck lymphadenopathy. The patient had noted a sudden onset of non-tender swelling of the lymph node, accompanied by an episode of high-grade fever. Abdominal ultrasonography demonstrated mild hepatomegaly and retroperitoneal lymphadenopathy. Laboratory studies demonstrated a decreased white blood cell count of 2300/mm^3. There were no atypical lymphocytes in peripheral blood. A moderately abnormal liver function was also noted. A right cervical lymph node biopsy was performed. Subsequent serologic tests for EBV gave the following results: a viral capsid antigen (VCA) IgG titer of 14.5 (< 0.5 mg/dl), a VCA IgM titer of 2.5 (< 0.5 mg/dl), an early antigen (EA) IgG titer of 5.6 (< 0.5 mg/dl), and an EBV nuclear antigen (EBNA) titer of 10.7 (< 0.5 mg/dl).

Case 3

A 35-year-old Japanese man presented with a two-month history of swelling in the right retroauricular region. Biopsy findings of the enlarged lymph node were tentatively diagnosed as reactive change of the lymph node. Subsequent laboratory studies demonstrated a white blood cell count of 5300/mm^3. There was no atypical lymphocytosis in the peripheral blood. Subsequent serologic tests for EBV gave the following results: a viral capsid antigen (VCA) IgG titer of 1:160, a VCA IgM titer of 1:10, an early antigen (EA) titer of less than 1:10, and an EBNA titer of less than 1:10. Serologic tests for *T. gondii* demonstrated the following results: a titer of *T.* IgM was 0.1 (< 0.8 IU/ml), and a titer of *Toxoplasma* IgG was 3 (< 6 IU/ml).

Materials and methods

Three-micrometer-thick sections were cut from formalin-fixed, paraffin-embedded tissues and stained with hematoxylin-eosin (HE) and Giemsa.

Immunohistochemical studies were performed using the antigen retrieval method on the basis of the avidin-biotinylated peroxidase method or Ventana automated (BenchMark™) stainer according to the manufacturer's instructions. We used a panel of antibodies against human immunoglobulin light chain (kappa and lambda) (Novocastra, Newcastle, UK), PS-1 (CD3; MBL, Nagoya, Japan), 4C7 (CD5; Novocastra), 56C6 (CD10; Novocastra), L26 (CD20; Dako, A/S, Glostrup, Denmark), 1B12 (CD23; Novocastra), DFT-1 (CD43;Dako), and SP4 (Cyclin D1; Nichirei Co., Tokyo, Japan). The primary antibodies were replaced by normal rabbit- and mouse-serum to establish a negative control.

In situ hybridization (ISH) with 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 (BenchMark™) stainer or using the hybridization kit (Dako).

Additional paraffin-embedded tissues from the biopsy specimen were available in two cases (nos. 1 and 2). We extracted the DNA from the paraffin-embedded tissue using a commercial extraction kit (TaKaRa DEXPAT). The specimens were prepared for polymerase chain reaction (PCR), and the presence or absence of *T. gondii* DNA was investigated using the PCR method [9]. Paraffin-embedded tissue definite for *T. gondii* infection served as a positive control.

Results

The size of the resected lymph nodes was as follows: Case 1 was 1.3 cm in diameter, Case 2 was 1.1 cm in diameter, and Case 3 was 1.5 cm in diameter. Histollogically, lymph node lesions of three cases were characterized by (i) numerous lymphoid follicles with hyperplastic germinal centers (Fig. 1); (ii) small clusters or single epithelioid histiocytes located within the hyperplastic germinal centers and periphery of lymphoid follicles, as well as interfollicular areas (Figs. 1 and 2a); and (iii) the presence of multiple foci of MBCs (Fig. 1). In two cases (nos. 1 and 3), a portion of the lymphoid follicles was surrounded by an incomplete pale ring of the marginal zone distribution pattern (MBCs) (Fig. 1). The pale ring was composed of MBCs with abundant pale clear cytoplasm. Cytologically, two types of MBCs were identified, as previously reported by Plank et al. [16]: (i) the common type of MBCs which are composed of medium-sized cells with irregular or bean-shaped nuclei and inconspicuous nucleoli (Fig. 2b); and (ii) the large cell type composed of largely transformed MBCs with large round nuclei demonstrating vesicular chromatin and prominent nucleoli (Fig. 2b). The common type of MBCs was predominant in two cases (nos. 2 and 3), while the large cell type was observed in one case (no. 1). Small lymphocytes, plasma cells, and histiocytes with or without epithelioid cell features were intermingled with MBCs. Immunohistochemical study demonstrated that MBCs were CD3-, CD5-, CD20+, CD43-, and bcl-2- (Fig. 2c). A small number of MBCs were polytypic intracytoplasmic immunoglobulins.

On in situ hybridization, numerous EBER-positive cells were demonstrated in the germinal center and in the interfollicular area in two cases (nos. 2 and 3) (Fig. 2d), whereas up to 100 EBER-positive cells were observed in the germinal center and the interfollicular area in Case 1.

Using PCR for B1 gene (producing a theoretical amplicon (96 nucleotides)), P30 gene (522 nucleotides), and 18S rDNA gene (88 nucleotides), we found that *Toxoplasmic gondii* DNA was absent in

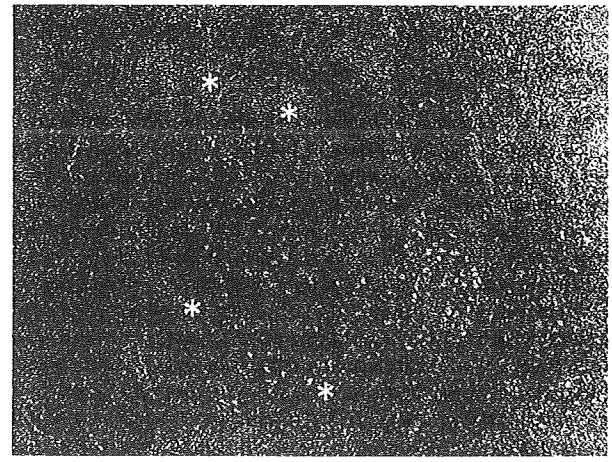

Fig. 1. Scanning magnification of the lesion. Note an enlarged lymphoid follicle with active germinal centers, clusters of epithelioid cells, and a lymphoid follicle was surrounded by an incomplete pale ring of MBCs (*). Note epithelioid cell clusters in a germinal center. Case 1 HE × 10.

Please cite this article as: M. Kojima, et al., Infectious mononucleosis lymphoadenitis showing histologic findings indistinguishable from toxoplasma lymphadenitis. A report of three cases, Pathol. Res. Practice (2010), doi:10.1016/j.prp.2009.12.010

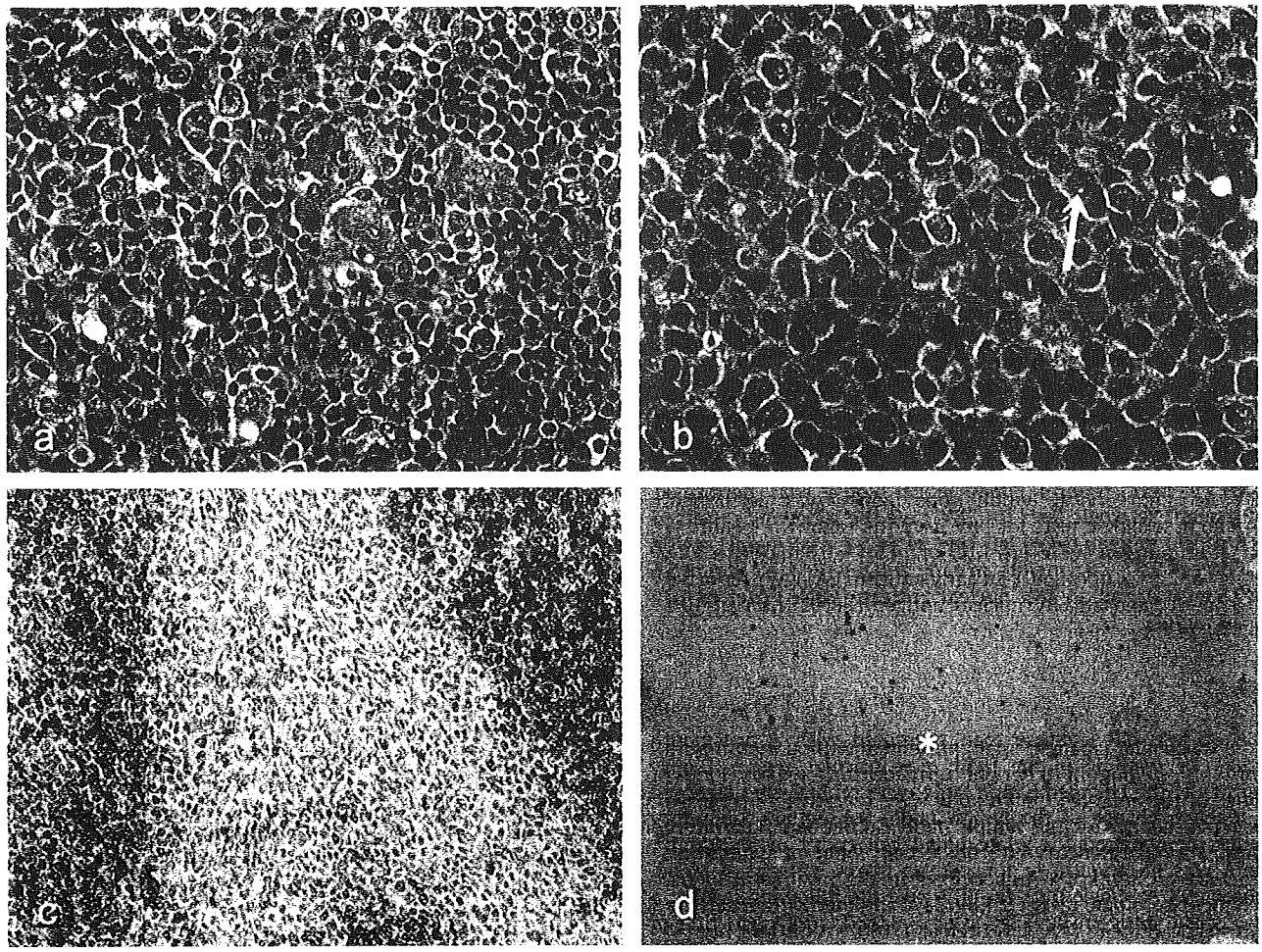

Fig. 2. (a) High power field of Fig. 1. Reactive germinal center contains tangible body macrophages and small clusters or single epithelioid cells (arrows). Case 1 (HE × 40). (b) High power field of Fig. 1. The common type of MBC was composed of medium-sized cells with irregular or bean-shaped nuclei and inconspicuous nucleoli with abundant clear cytoplasm (black arrow). The large cell type was composed of largely transformed MBCs with large round nuclei demonstrating vesicular chromatin and prominent nucleoli (white arrow). Case 1 (HE × 60). (c) Both types of MBCs were bcl-2- Case (3 × 20). (d) Numerous EBER+ cells were located in the germinal center (*) and the interfollicular area. Case 2 ISH × 10 counterstain, nuclear fast red.

two lesions (nos. 1 and 2) examined. *T. gondii* DNA; B1 gene, P30 gene, and 18S rDNA gene were detected in the positive control.

Discussion

IM is an acute lymphoproliferative disorder (LPD) that typically occurs in young patients and is usually caused by primary EBV infection [4,7,19]. The diagnosis of IM is usually based on clinical and serologic findings [6]. However, a lymph node biopsy may be performed when malignant lymphoma is a clinical consideration in patients with an atypical clinical feature [2,11,17,18]. Atypical features include age over 30 years, a negative heterophil antibody titer, and the absence of atypical lymphocytosis in peripheral blood and lymphadenopathy without fever or sore throat [2,11,7,18]. Two of our three cases showed atypical clinical presentations, including an absence of atypical lymphocytosis in peripheral blood in two cases (nos. 2 and 3), an age of 35 years in Case 3, and an absence of systemic symptoms in Case 3. As a result, these two cases underwent lymph node biopsy to exclude malignant lymphoma.

In healthy adults, the most common manifestation of toxoplasmosis is localized lymphadenopathy [3,5,8,10,12]. The posterior cervical lymph nodes are most commonly and characteristically involved [3,10,12]. Patients may be entirely asymptomatic or demonstrate mild, nonspecific symptoms, such as fever and myalgia. However, occasionally, generalized lymphadenopathy, sometimes accompanied by moderate hepatosplenomegaly, may occur [3,10,12]. It appears that a portion of toxoplasmic lymphadenitis shows clinical findings similar to those of IM in some aspects.

The histologic findings of the three cases presented here showed a histologic triad of toxoplasma lymphadenitis, including numerous lymphoid follicles with hyperplastic germinal centers, small clusters or single epithelioid histiocytes, and multiple foci of MBCs [8,15]. Moreover, all three lesions contained isolated or small clusters of epithelioid histiocytes within hyperplastic germinal centers and the periphery of lymphoid follicles, as well as the interfollicular area, which is the most specific histologic finding in toxoplasmic lymphadenitis [8,15].

However, the serologic findings confirmed EBV infection in all three cases [6]. Moreover, *T. gondii* infection was excluded in at least one case (no. 3) based on serologic findings [3]. Using PCR, Lin et al. demonstrated *T. gondii* DNA in 10 of 12 lesions s[illegible]g a histopathologic triad of toxoplasmic lymphadenitis [15]. They concluded that the PCR method shows highly s[illegible] findings for the diagnosis of toxoplasmic lymphadenitis [1[illegible]owever, PCR

Please cite this article as: M. Kojima, et al., Infectious mononucleosis lymphoadenitis showing histologic findings indistinguishable from toxoplasma lymphadenitis. A report of three cases, Pathol. Res. Practice (2010), doi:10.1016/j.prp.2009.12.010

analyses demonstrated that there was no *T. gondii* DNA in either of the two lesions (nos. 1 and 2). On ISH, EBER+ lymphocytes were detected in all three lesions. Clinical and EBV findings indicated that the present three cases were IM.

Interestingly, a portion of the lymphoid follicles were surrounded by a pale rim of the MBCs in two lesions (marginal zone distribution pattern) [1]. These histologic findings appear to be similar to those of nodal marginal zone B-cell lymphoma [1]. However, there were no CD43+ or bcl-2+ MBCs in any of the lesions [1]. A small number of MBCs had polytypic intracytoplasmic immunoglobulins. The immunohistochemical findings indicated that MBCs were not neoplastic.

It appears that previous descriptions emphasize the differential diagnostic problems between IM lymphadenitis and malignant lymphomas [2,7,11,17,18]. The three cases presented here clearly demonstrated that a portion of IM showed histologic findings indistinguishable from toxoplasma lymphadenitis. From a therapeutic perspective, it is important to discriminate IM lymphadenitis from toxoplasmic lymphadenitis particularly in cases showing atypical clinical features.

Finally, it may be important for pathologists to bear in mind that not every patient with serologic evidence of EBV infection shows clinical findings of IM, and not every lymph node biopsied in a patient with serologic evidence of EBV infection shows changes due to IM [3,7,19].

Uncited reference

Q1 [14].

Acknowledgment

This work was supported by the Intractable Diseases, the Health and Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare (H21-112)

References

[1] E. Campo, R. Miquel, L. Krenacs, L. Sorbara, M. Raffeld, E.S. Jaffe, Primary nodal marginal zone lymphoma of splenic and MALT type, Am. J. Surg. Pathol. 23 (1999) 59–68.

[2] C.C. Childs, D.M. Parham, C.W. Berard, Infectious mononucleosis. The spectrum of morphologic changes simulating lymphoma in lymph node and tonsils, Am. J. Surg. Pathol. 11 (1987) 122–132.

[3] R.A. Durlach, K. Kaufer, L. Carral, J. Hirt, Toxoplasmic lymphadenitis - clinical and serological profile, Clin. Microbiol. Infect. 9 (2003) 625–631.

[4] N.F.C. Gowing, Infectious mononucleosis: histopathologic aspects, Pathol. Annu. 10 (1975) 1–20.

[5] G.F. Gray Jr., A.C. Kimball, B.H. Kean, The posterior cervical lymph node in toxoplasmosis, Am. J. Pathol. 69 (1972) 349–358.

[6] W. Henle, G.E. Henle, C.A. Horwiltz, Epstein-Barr virus specific diagnostic tests in infectious mononucleosis, Hum. Pathol. 5 (1974) 551–565.

[7] H.L. Ioachim, L.J. Medeiros, Ioachim's Lymph Node Pathology, fourth ed., Lippincott, Williams & Wilkins, Philadelphia, 2009 (p. 76–82).

[8] H.L. Ioachim, L.J. Medeiros, Ioachim's Lymph Node Pathology, fourth ed., Lippincott, Williams & Wilkins, Philadelphia, 2009 (p. 159–164).

[9] C.D. Jones, N. Okhravi, P. Adamson, S. Tasker, S. Lightman, Comparison of PCR detection methods for B1, P30, and18S rDNA genes of *T. gondii* in aqueous humor, Invest. Ophthalmpl. Vis. Sci. 41 (2000) 634–644.

[10] T.C. Jones, B.H. Kean, A.C. Kimball, Toxoplasmic lymphadenitis, JAMA 192 (1965) 87–91.

[11] M. Kojima, M. Kashimura, H. Itoh, A. Noro, B. Akikusa, M. iijima, K. Shimizu, N. Masawa, S. Nakamura, Epstein-Barr virus related reactive lymphorpoiferative disorders in middle-aged or elderly patients presenting with atypical features. A clinicopathological study of six cases, Pathol. Res. Pract. 203 (2007) 587–591.

[12] J.A. Krick, J.S. Remington, Current concepts in parasitology - toxoplasmosis in the adult: an overview, N. Eng. J. Med. 298 (1978) 550–553.

[13] R. Lai, L.M. Weiss, K.L. Chang, D.A. Arber, Frequency of CD43 expression in non-Hodgkin lymphoma. A survey of 742 cases and further characterization of rare CD43+ follicular lymphomas, Am. J. Clin. Pathol. 111 (1999) 488–494.

[14] M.-H. Lin, T.-t. Kuo, Specificity of the histopathological triad for the diagnosis of toxoplasmic lymphadenitis: polymerase chain reaction study, Pathol. Int. 51 (2001) 619–623.

[15] M. Miettinen, Histological differential diagnosis between lymph node toxoplasmosis and other benign lymph node hyperplasia, Histopathology 5 (1981) 205–216.

[16] L. Plank, M.-L. Hansmann, R. Fischer, The cytological spectrum of the monocytoid B-cell reaction: recognition of its large cell type, Histopathology 23 (1993) 425–431.

[17] S.S. Shin, G.J. Berry, L.M. Weiss, Infectious mononucleosis. Diagnosis by in situ hybridization in two cases with atypical features, Am. J. Surg. Pathol. 15 (1991) 625–631.

[18] J.G. Strickler, F. Fedeli, C.A. Horwitz, C.M. Copenhaver, G. Frizzera, Infectious mononucleosis in lymphoid tissue-histopathology, in situ hybridization and differential diagnosis, Arch. Pathol. Lab. Med. 117 (1993) 269–278.

[19] W.St.C. Symmers, Infectious mononucleosis, in: K. Henry, W.St.C. Symmers (Eds.), Thymus, Lymph Nodes, Spleen and Lymphatics. Systemic Pathology. third ed. vol 7. Churchill Livingstone, Edinburgh, 1992, p. 432–437.

[20] T. Wang, J. Lasota, C.A. Hanau, M. Miettinen, Bcl-2 oncoprotein is widespread in lymphoid tissue and lymphoma but its different expression in benign versus malignant follicles and monocytoid B-cell proliferation is of diagnostic value, APMIS 103 (1995) 55–62.

Please cite this article as: M. Kojima, et al., Infectious mononucleosis lymphoadenitis showing histologic findings indistinguishable from toxoplasma lymphadenitis. A report of three cases, Pathol. Res. Practice (2010), doi:10.1016/j.prp.2009.12.010

Original Paper

Biliary Lesions Associated with Autoimmune Pancreatitis

Terumi Kamisawa[1], Kensuke Takuma[1], Hajime Anjiki[1], Naoto Egawa[1], Masanao Kurata[2], Goro Honda[2], Kouji Tsuruta[2], Atsutake Okamoto[2]

[1]Department of Internal Medicine, [2]Department of Surgery, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

Corresponding Author: Terumi Kamisawa, MD, Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan.
Tel: +81-3-3823-2101, Fax: +81-3-3824-1552, E-mail: kamisawa@cick.jp

KEY WORD:
Autoimmune pancreatitis; Sclerosing cholangitis; Sclerosing cholecystitis; IgG4; Primary sclerosing cholangitis

ABBREVIATIONS:
Autoimmune Pancreatitis (AIP); Primary Sclerosing Cholangitis (PSC); Ultrasonography (US); Computed Tomography (CT); Endoscopic Retrograde Cholangiopancreatography (ERCP); Magnetic Resonance Cholangiopancreatography (MRCP); Endoscopic Ultrasonography (EUS); Intraductal Ultrasonography (IDUS)

ABSTRACT

Background/Aims: Characteristic radiological features of biliary lesions in patients with autoimmune pancreatitis (AIP) have not yet been identified.

Methodology: Bile duct lesions and their relationships to other clinical findings were assessed in 43 AIP patients.

Results: Of the 43 AIP patients, 34 (79%) had bile duct stenosis. In all the 34 patients, the lower bile duct was involved; in 21 of these, only the lower bile duct was involved, and in 13 patients, there was widespread wall thickening of the middle and upper bile duct where stenosis was not obvious on cholangiography. Furthermore, 4 patients with extensive bile duct involvement also had stenosis of the intrahepatic bile duct. All patients with bile duct involvement had involvement of the head portion of the main pancreatic duct. None of the 6 patients with involvement of only the body and/or tail portion of the main pancreatic duct had bile duct involvement. Gallbladder wall thickening was more frequently noted in patients with extensive bile duct involvement ($p<0.01$). Serum IgG4 levels were significantly more elevated in patients with extensive bile duct involvement ($p<0.05$).

Conclusions: AIP patients with extensive bile duct involvement characterized by widespread wall thickening of the bile duct may have more active disease.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a recently described clinical entity in which the pathogenesis may involve autoimmune mechanisms. AIP has several characteristic features, such as elderly male preponderance, elevation of serum gammaglobulin or IgG levels, especially IgG4, presence of autoantibodies, and a favorable response to steroid therapy. It is also characterized radiologically by enlargement of the pancreas and irregular narrowing of the main pancreatic duct, and histologically by dense lymphoplasmacytic infiltration and fibrosis of the pancreas. Another characteristic finding is the frequent association between AIP and various extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialadenitis, and retroperitoneal fibrosis (1-4).

Bile duct stenosis frequently occurs with AIP, and the major initial symptom of AIP is obstructive jaundice due to this stenosis. The stenotic portion of the bile duct is usually the lower bile duct, though stenosis of the intrahepatic bile duct is sometimes detected. When stenosis is found in the intrahepatic bile duct, the cholangiographic appearance is very similar to that of primary sclerosing cholangitis (PSC) (5,6). Furthermore, swelling of the gallbladder wall is also occasionally detected in AIP patients (4,7). Although there are several reports dealing with sclerosing cholangitis associated with AIP (5,8,9), the characteristic radiological features of the biliary lesions have not been identified, and their relationships to other clinical findings in AIP patients have not yet been determined. In this study, bile duct lesions seen in AIP patients were divided into three groups (no involvement, involvement of only the lower bile duct, and extensive involvement of the bile duct), and their relationships to other clinical parameters that might predict the disease course or outcome in AIP patients were assessed.

METHODOLOGY

Patients

Between 1980 and 2007, 43 patients (36 males and 7 females, average age 66.4 years) were diagnosed with AIP based on the following clinicopathological criteria: irregular narrowing of the main pancreatic duct on endoscopic retrograde pancreatography (n=43), pancreatic enlargement on ultrasonography (US) or computed tomography (CT) (n=42), presence of autoantibodies (n=22), elevated serum IgG4 in excess of 135 mg/dl (n=31), characteristic histological findings in the pancreas (n=12), and responsiveness to steroid therapy (n=29). All patients underwent endoscopic retrograde cholangiopancreatography (ERCP), CT, and US; 20 patients also underwent magnetic resonance cholan-

Hepato-Gastroenterology 2009; 56:1190-1193

giopancreatography (MRCP).

Radiological evaluation

The stenotic portion of the bile duct was examined by ERCP and/or MRCP, and wall thickening of the bile duct in which stenosis was not obvious on cholangiography was assessed on CT and US. Bile duct involvement was classified into three groups: no involvement (Group A), involvement of only the lower bile duct (Group B, **Figure 1**), and extensive involvement of the bile duct (Group C). Extensive involvement of the bile duct was defined as stenosis of the intrahepatic bile duct with lower bile duct stenosis, or widespread wall thickening of the middle and upper bile duct where stenosis was not obvious on cholangiography in addition to stenosis of the lower bile duct (**Figure 2**). Gallbladder wall thickening (more than 4mm) was assessed on US (**Figure 3**). Involvement of the main pancreatic duct was categorized into involvement of the head portion of the main pancreatic duct, including the diffuse type, and involvement of only the body and/or tail portion. Extrapancreatic lesions, including sclerosing sialadenitis and retroperitoneal fibrosis, were evaluated on physical examination, CT, and US.

Statistical analysis

In the three groups categorized according to biliary involvement, differences in age and serum IgG4 levels were analyzed first by the Kruskal-Wallis H-test, followed by Mann-Whitney's U-test if significant. The other analyses were performed using Fisher's exact test. In all tests, corrected p values of <0.05 were considered statistically significant.

RESULTS

Bile duct involvement was absent in 9 patients (Group A), only the lower bile duct was involved in 21 patients (Group B), and extensive bile duct involvement was seen in 13 patients (Group C). Four group C patients had intrahepatic bile duct stenosis. A beaded appearance with diffusely distributed short and annular strictures of the bile duct, which is commonly seen in PSC, was not detected in any of the AIP patients. Left-sided deviation of the lower bile duct, which is frequently detected in patients with pancreatic head cancer, was detected in only 17 (40%) AIP patients.

In all of the AIP groups, the patients were predominantly elderly males. All patients with bile duct involvement also had involvement of the head portion of the main pancreatic duct. However, none of the 6 patients with involvement of only the body and/or tail portion of the main pancreatic duct had bile duct involvement. Gallbladder wall thickening occurred more frequently in patients with extensive bile duct involvement ($p<0.01$). Serum IgG4 levels were significantly more elevated in patients with extensive bile duct involvement than in those with only lower bile duct involvement ($p<0.05$). Similar proportions of patients in each group had involvement of other organs, such as sclerosing sialadenitis and retroperitoneal fibrosis (**Table 1**).

In the 29 AIP patients (Group A: n=5, Group B: n=15, and Group C: n=9) who were treated with steroid, all biliary and pancreatic lesions responded well; however, one group C patient had a recurrent biliary lesion and another group C developed ir-

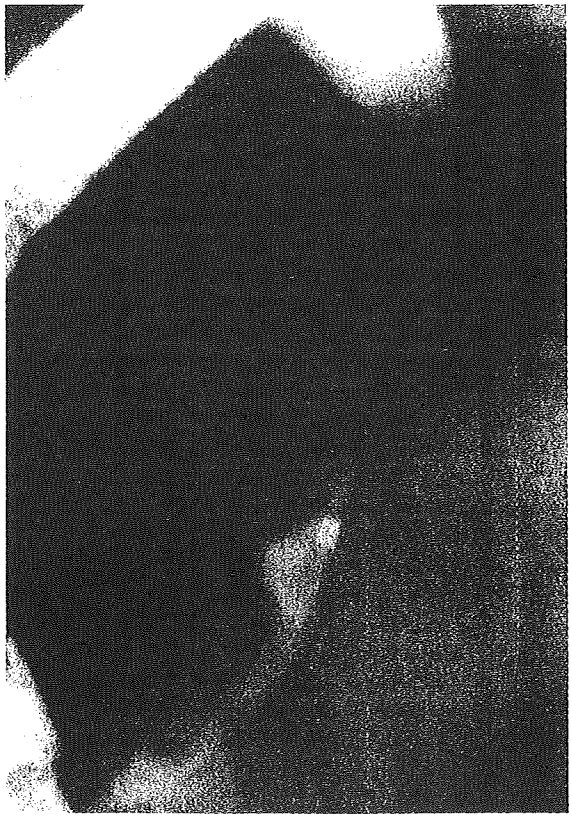

FIGURE 1 Endoscopic retrograde cholangiography of autoimmune pancreatitis patient showing stenosis of the lower bile duct

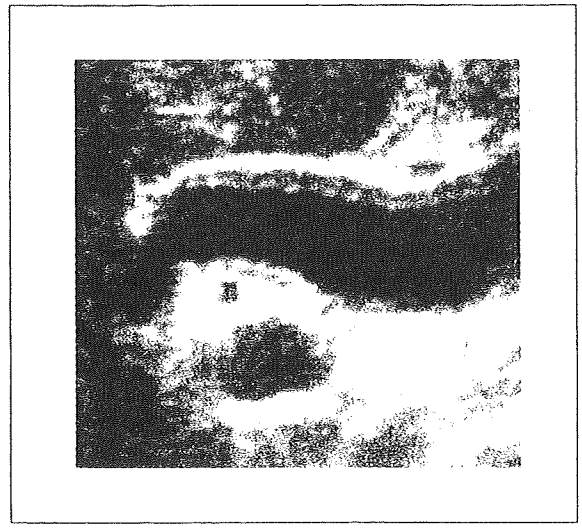

FIGURE 2 Ultrasonography of autoimmune pancreatitis patient showing widespread wall thickening of the middle and upper bile duct where stenosis is not obvious on cholangiography

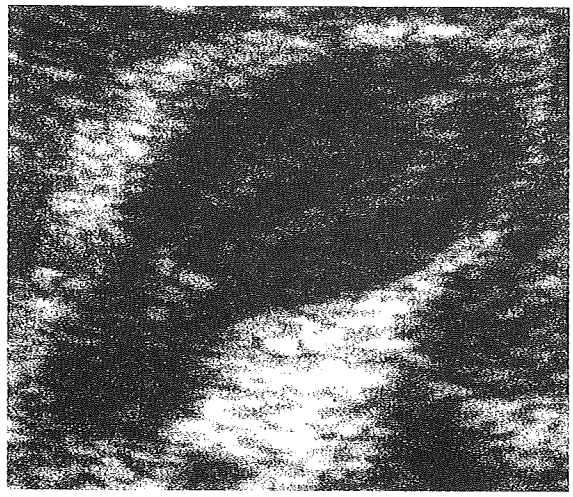

FIGURE 3 Ultrasonography of autoimmune pancreatitis patient showing gallbladder wall thickening

regular dilatation of the main pancreatic duct. Six patients underwent pancreatoduodenectomy and five patients underwent choledochojejunostomy or choledochoduodenostomy with pancreatic biopsy on suspicion of pancreatic cancer. The other 3 patients were followed up conservatively.

DISCUSSION

PSC is a progressive disease of unknown etiology that involves the intra- and extrahepatic bile ducts. The characteristic findings on cholangiography include a beaded appearance with diffusely distributed short and annular strictures of the bile duct. Regardless of therapy, PSC sometimes leads to liver cirrhosis, and liver transplantation may be the only effective treatment (10). On the other hand, bile duct stenosis occurs frequently with AIP, and AIP patients' major initial symptom is obstructive jaundice (1-4). When AIP patients develop stenosis in the intrahepatic bile duct, the cholangiographic appearance is very similar to that of PSC (5,6). However, since AIP responds well to steroid therapy, it is necessary to discriminate between these two diseases before making a therapeutic decision.

In the present study, bile duct stenosis was detected in 34 (79%) of 43 AIP patients. All 34 patients with bile duct stenosis had lower bile duct stenosis; 21 of these had only lower bile duct involvement, while 13 had widespread wall thickening of the middle and upper bile duct where stenosis was not obvious on cholangiography. Furthermore, 4 patients with extensive bile duct involvement also had stenosis of the intrahepatic bile duct.

On histological examination of the specimens obtained from pancreatoduodenectomies of the AIP patients, the wall of the stenotic bile duct was found to be thickened by marked fibrosis, with dense infiltration of IgG4-positive plasma cells

TABLE 1 Relationship Between Biliary Involvement and Other Clinical and Radiological Findings in Autoimmune Pancreatitis Patients

Biliary involvement	Age at diagnosis (years)	Male/Female	P-duct Head/Body+Tail	Gallbladder wall thickening +/-	Serum IgG4 level (mg/dl)	Other organ involvement +/-
Group A None (n=9)	68.0 (64.0-75.0)	6/3	3/6*1	0/9	240.0 (138.0-458.7)	4/5
Group B Lower bile duct only (n=21)	67.0 (59.0-73.5)	19/2	21/0	4/17	208.0 (125.8-355.0)	4/17
Group C Extensive bile duct (n=13)	66.0 (64.5-70.0)	10/3	13/0	9/4*2	450.0*3 (310.0-1095.0)	6/7

Age at diagnosis and serum IgG4 levels are indicated as median (quartile).
P-duct means lesion involving the main pancreatic duct.
Other organ involvement included sclerosing sialadenitis and retroperitoneal fibrosis.
*1: $p<0.01$ compared with groups 2 and 3
*2: $p<0.01$ compared with groups 1 and 2
*3: $p<0.05$ compared with group

and lymphocytes similar to the findings noted in the pancreas. This fibroinflammatory change was compatible with sclerosing cholangitis and was detected extensively in the wall of almost the whole bile duct, though the degree of the inflammation differed among the cases (3, 4, 11, 12). Hyodo *et al.* (13) and Hirano *et al.* (9) reported that wall thickening of the bile duct in which stenosis was not obvious on cholangiography could sometimes be detected on endoscopic ultrasonography (EUS) or intraductal ultrasonography (IDUS). Nakazawa *et al.* (14) reported that, on cholangiography, stenosis of the bile duct was detected in 34 (92%) of 37 AIP patients; 18 had stenosis of only the lower bile duct, and 3 had a stricture in only the hepatic hilar lesion. In cases in which stenosis is detected in the intrahepatic bile duct, the presence of stenosis of the lower bile duct and wall thickening of the bile duct with no obvious stenosis on cholangiography seems to be useful for differentiating AIP from cholangiocarcinoma and PSC.

Interestingly, sclerosing cholangitis was found in 33 (89%) of 37 AIP patients with pancreatic head involvement, but it was not found in any of the 6 AIP patients with segmentally restricted involvement in the pancreatic body and/or tail. Although EUS and IDUS demonstrated that stenosis and wall thickening of the lower bile duct in AIP patients was the result of concentric change itself and not caused by extrinsic compression secondary to inflammatory pancreatic tissue (13), fibroinflammation of the pancreas around the lower bile duct may also be a contributing factor.

Gallbladder wall thickening, occasionally detected in AIP patients, is found on histological examination to be sclerosing cholecystitis that is composed of transmural fibrosis with dense IgG4-positive plasma cells and lymphocytes in the gallbladder wall (7). Patients with extensive bile duct involvement more frequently have gallbladder wall thickening and higher serum IgG4 levels. Hamano *et al.* (15) reported that AIP patients with several extrapancreatic lesions had higher serum IgG4 levels than those with few lesions; this suggested that, compared to AIP patients with few lesions, AIP patients with many extrapancreatic lesions may have more active disease. In the present study, most AIP patients responded well to steroid therapy; however, one patient with extensive bile duct involvement developed recurrence, and another patient with extensive bile duct involvement had poor resolution of the main pancreatic duct lesion. Based on these findings, AIP disease activity may be high in patients with extensive bile duct involvement.

In conclusion, the lower bile duct was frequently involved in AIP patients. AIP patients with extensive bile duct involvement who have widespread wall thickening of the middle and upper bile duct frequently have gallbladder wall thickening and higher serum IgG4 levels; these patients may have more active disease.

REFERENCES

1. **Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N:** Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig Dis Sci 1995; 40:1561-1568
2. **Okazaki K, Chiba T:** Autoimmune-related pancreatitis. Gut 2002; 51: 1-4
3. **Kamisawa T, Egawa N, Nakajima H, Tsuruta K, Okamoto A:** Extrapancreatic lesions in autoimmune pancreatitis. J Clin Gastroenterol 2005; 39:904-907
4. **Kamisawa T, Okamoto A:** Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. J Gastroenterol 2006; 41:613-625
5. **Nakazawa T, Ohara H, Yamada T, et al:** Atypical primary sclerosing cholangitis cases associated with unusual pancreatitis. Hepatogastroenterology 2001; 48:625-630
6. **Kamisawa T, Egawa N, Tsuruta K, Okamoto A, Funata N:** Primary sclerosing cholangitis may be overestimated in Japan. J Gastroenterol 2005; 40:318-319
7. **Kamisawa T, Tu Y, Nakajima H, et al:** Sclerosing cholecystitis associated with autoimmune pancreatitis. World J Gastroenterol 2006; 12:3736-3739
8. **Nakazawa T, Ohara H, Yamada T, et al:** Cholangiography can discriminate sclerosing cholangitis with autoimmune pancreatitis from primary sclerosing cholangitis. Gastrointest Endosc 2004; 60:937-944
9. **Hirano K, Shiratori Y, Komatsu Y, et al:** Involvement of the biliary system in autoimmune pancreatitis: a follow-up study. Clin Gastroenterol Hepatol 2003; 1:453-464
10. **Wiesner RH, Grambsch PM, Dickson ER, et al:** Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology 1989; 10:430-436
11. **Kamisawa T, Funata N, Hayashi Y, et al:** A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol 2003; 38:982-984
12. **Kamisawa T, Funata N, Hayashi Y, et al:** Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis. Gut 2003; 52:683-687
13. **Hyodo N, Hyodo T:** Ultrasonographic evaluation in patients with autoimmune-related pancreatitis. J Gastroenterol 2003; 38:1155-1161
14. **Nakazawa T, Ohara H, Sano H, et al:** Difficulty in diagnosing autoimmune pancreatitis by imaging findings. Gastrointest Endosc 2007; 65:99-108
15. **Hamano H, Arakura N, Muraki T, Ozaki Y, Kiyosawa K, Kawa S:** Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis. J Gastroenterol 2006; 41:1197-1205

Allergic manifestations in autoimmune pancreatitis

Terumi Kamisawa[a], Hajime Anjiki[a], Naoto Egawa[a] and Naoko Kubota[b]

Objective Autoimmune pancreatitis (AIP) is a peculiar type of pancreatitis of presumed autoimmune etiology. However, target antigens for AIP have not been detected. Furthermore, its preponderant occurrence in elderly men and the very dramatic response with no major residual deformity to oral steroid therapy within 2 weeks suggest that the pathogenesis of AIP might not involve autoimmune mechanism. Recently, it was reported that the immune reaction closely involved in the pathogenesis of allergic disorders was upregulated in the affected tissues of AIP. This study aimed to examine the allergic manifestations in AIP patients.

Methods This case series consisted of 45 AIP patients. Present and/or past histories of allergic diseases, clinical findings, and laboratory data were reviewed.

Results Twenty patients (allergic-type AIP) had histories of allergic diseases, such as acute allergic rhinitis ($n=11$), including rose and hay fever, atopic dermatitis ($n=5$), bronchial asthma ($n=3$), drug allergy ($n=2$), and hypersensitivity pneumonitis ($n=1$). Serum IgE levels were elevated in only 12 allergic-type AIP patients, and they were significantly higher in allergic-type AIP patients than in AIP patients without allergic manifestations ($P=0.0001$). The peripheral eosinophil count was significantly elevated in allergic-type AIP patients ($P=0.048$). With respect to initial symptoms, obstructive jaundice was less frequent in allergic-type AIP patients ($P=0.012$), and abdominal pain was detected in only five patients with allergic-type AIP ($P=0.013$). Allergic-type AIP occurred predominantly from September to February in 19 of 20 patients ($P=0.001$).

Conclusion Allergic manifestations were detected in about half of the AIP patients, and allergic mechanisms may be related to the occurrence of AIP in these patients. *Eur J Gastroenterol Hepatol* 21:1136–1139 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

European Journal of Gastroenterology & Hepatology 2009, 21:1136–1139

Keywords: allergy, autoimmune pancreatitis, eosinophil, IgE, IgG4

Department of [a]Internal Medicine and [b]General Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

Correspondence to Terumi Kamisawa, MD, PhD, Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan
Tel: +81 3 3823 2101; fax: +81 3 3824 1552; e-mail: kamisawa@cick.jp

Received 25 November 2008 Accepted 18 January 2009

Introduction

Autoimmune pancreatitis (AIP) is a peculiar type of pancreatitis of presumed autoimmune etiology, and it has become a distinct entity that is recognized worldwide [1–5]. In 1995, Yoshida *et al.* [6] first proposed the concept of AIP from analysis of their case and the 10 other reported cases, and they summarized the clinical features as follows: (i) increased serum γ-globulin or IgG levels; (ii) presence of autoantibodies; (iii) effectiveness of steroid therapy; (iv) occasional association with other autoimmune diseases, such as Sjogren's syndrome; (v) diffuse enlargement of the pancreas; (vi) diffuse irregular narrowing of the main pancreatic duct on endoscopic retrograde pancreatography; (vii) fibrotic change with lymphocyte infiltration histopathologically; (viii) mild symptoms, usually without acute attacks of pancreatitis; (ix) occasional association with stenosis of the lower bile duct, cholestatic liver dysfunction, and hyperbilirubinemia; (x) no pancreatic calcification; and (xi) no pancreatic cysts. Based on the first four findings, they suspected that the cause of the pancreatitis was an autoimmune mechanism, and named this pancreatitis as AIP.

However, target antigens for AIP have not been detected. On the basis of histological and immunohistochemical examinations of various organs and extrapancreatic lesions of AIP patients, AIP might be a pancreatic lesion of an IgG4-related systemic disease, and extrapancreatic lesions occasionally associated with AIP might not be a part of an autoimmune disease but organs involved in this systemic disease [5,7,8]. Furthermore, its preponderant occurrence in elderly men and the very dramatic response with no major residual deformity to oral steroid therapy within 2 weeks [1–5] suggest that the pathogenesis of AIP might not involve an autoimmune mechanism. Recently, it was shown that the expressions of Th2 cytokines [IL-4, IL-5, and IL-13] and regulatory cytokines (IL-10 and transformation growth factor-β) were upregulated in the affected tissues of AIP [9]. The immune reactions that are predominantly mediated by Th2 cells and regulatory T cells are not common in classical autoimmune diseases, but they are closely involved in the pathogenesis of allergic disorders such as bronchial asthma and atopic dermatitis [10].

0954-691X © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins

DOI: 10.1097/MEG.0b013e3283297417

Thus, we studied allergic manifestations in 45 AIP patients and compared the clinical features of AIP patients with and without allergic manifestations.

Patients and methods

Patients

Between April 1990 and July 2008, 48 patients were consecutively diagnosed with AIP based on the Asian diagnostic criteria for AIP [11]. All patients showed pancreatic enlargement and irregular narrowing of the main pancreatic duct. Forty patients were also diagnosed based on the revised clinical diagnostic criteria of AIP 2006 [12] in Japan, and the other eight patients were diagnosed based on the imaging criterion and good responsiveness to steroid therapy.

Methods

Allergy clinic charts of the patients were reviewed for present and/or past histories of allergic diseases, such as acute allergic rhinitis, atopic dermatitis, and bronchial asthma. Of 48 AIP patients, three patients whose allergy clinic charts were not fully described were excluded from this study. They were treated before 1995 and were not followed up. Finally, 45 AIP patients (37 men and 8 women, median age 67 years) were enrolled in this study. Authors confirmed allergic histories by interview in 36 followed up patients again.

Clinical findings and laboratory data (antinuclear antigen in 45 patients, rheumatoid factor in 38 patients, serum IgG4 level in 41 patients, serum IgE level in 35 patients, and peripheral eosinophil count in 45 patients) were also reviewed. Peripheral blood eosinophilia was identified when the absolute eosinophil count was greater than 600 cells/mm^3 [13]. This study was approved by the institutional review board of Tokyo Metropolitan Komagome Hospital.

Statistical analysis

Statistical analysis was performed using Wilcoxon's rank-sum test and Fisher's exact test. A P value of less than 0.05 was considered statistically significant.

Results

Of the 45 AIP patients, 20 had present and/or past histories of allergic diseases, such as acute allergic rhinitis (n=11), including rose and hay fever, atopic dermatitis (n=5), bronchial asthma (n=3), drug allergy (n=2), and hypersensitivity pneumonitis (n=1). The clinical features of the 20 AIP patients with allergic manifestations (allergic-type AIP) and the 25 AIP patients without allergic manifestations (nonallergic-type AIP) were compared.

There were no significant differences between the two types: in age at diagnosis, sex ratio, serum IgG4 levels, positivity of autoantibodies, and frequency of associated extrapancreatic lesions, such as sclerosing sialadenitis and retroperitoneal fibrosis. Elevated serum IgG4 levels were detected in 16 (84%) of 19 allergic-type AIP patients, and in 18 (82%) of 22 nonallergic-type AIP patients. Serum IgE levels (normal, 580 IU/ml) were elevated in 12 (80%) of 15 allergic-type AIP patients, and in none of 20 nonallergic-type AIP patients ($P < 0.0001$). Serum IgE levels were significantly higher in allergic-type AIP patients than in nonallergic-type AIP patients (P=0.0001). Peripheral blood eosinophilia was identified in five (25%) of allergic-type AIP patients (600–1390 cells/mm^3), and in none of nonallergic-type AIP patients (P=0.013). The peripheral eosinophil count was significantly elevated in allergic-type AIP patients (P=0.048). With respect to initial symptoms, obstructive jaundice was less frequent in allergic-type AIP patients (P=0.012), and abdominal pain was detected in only five patients with allergic-type AIP (P=0.013).

Allergic-type AIP occurred predominantly from September to February in 19 of 20 patients, whereas nonallergic-type AIP occurred from September to February in 12 of 25 patients (P=0.009) (Table 1).

Discussion

The concept of AIP was first proposed based on the frequent increases in serum IgG, the presence of autoantibodies, and responsiveness to steroid therapy [6]. However, target antigens for AIP have not been detected.

Table 1 **Clinical differences between autoimmune pancreatitis patients with and without allergic aspects**

	With allergic aspects (n=20)	Without allergic aspects (n=25)	P value
Age at diagnosis[a] (years)	65 (54.25–69.50)	68 (61.00–76.00)	0.166
Male/female	14/6	23/2	0.113
Obstructive jaundice +	9 (45%)	19 (76%)	0.062
Abdominal pain +	5 (25%)	0 (0%)	0.012
Autoantibody +	3 (15%)	6 (24%)	0.709
Serum IgG4[a] (mg/dl)	298.00 (198.00–450.00)	368 (125.75–796.00)	0.647
Serum IgE[a] (IU/ml)	793.50 (310.75–1288.00)	176 (98.500–221.00)	0.00001
Eosinophils[a] (cells/mm^3)	292.50 (106.00–582.00)	160 (104.50–284.00)	0.048
Extrapancreatic lesion +	5 (25%)	8 (32%)	0.744
On-set month, March–August/September–February	2/18	12/13	0.0009

+, present.
[a]Median (quartile range).

Its preponderant occurrence in elderly men and the very dramatic response with no major residual deformity to oral steroid therapy within 2 weeks [1–5] suggest that the pathogenesis of AIP might not involve an autoimmune mechanism. Although it has been reported that Sjogren's syndrome or primary sclerosing cholangitis is occasionally associated with AIP [14,15], it has become obvious that the salivary gland lesion in AIP is not Sjogren's syndrome but sclerosing sialadenitis with infiltration of abundant IgG4-positive plasma cells, and the bile duct lesion in AIP is not primary sclerosing cholangitis but sclerosing cholangitis with infiltration of abundant IgG4-positive plasma cells [16–18]. It has also been recognized that AIP might be a pancreatic lesion of an IgG4-related systemic disease, and extrapancreatic lesions occasionally associated with AIP might not be a part of an autoimmune disease but organs involved in this systemic disease [5,7,8]. Elevation of serum IgG4 concentrations is detected in 67–95% of AIP patients [19,20], and a close relationship between IgG4 and some allergic diseases has been reported [21]. Zen *et al.* [9] demonstrated that the expressions of Th2 cytokines and regulatory cytokines were upregulated in the affected tissues of AIP. The immune reactions that are predominantly mediated by Th2 cells and regulatory T cells are not common in classical autoimmune diseases, but they are closely involved in the pathogenesis of allergic disorders such as bronchial asthma and atopic dermatitis [10]. These findings led us to this study of allergic manifestations in AIP patients.

This study showed that 44% of AIP patients had allergic manifestations, and these allergic-type AIP patients frequently showed elevated serum IgE levels and peripheral eosinophil counts. According to a nationwide survey of AIP in Japan [22], an elevated peripheral eosinophil count (>500 cells/mm^3) was detected in 21% of AIP patients. Ito *et al.* [23] reported three AIP patients who had mild peripheral eosinophilia (less than 696 cell/mm^3). Sasahira *et al.* [24] reported an AIP patient showing peripheral eosinophilia (1343 cells/mm^3) who was associated with sclerosing cholangitis and inflammatory pseudotumor of the liver with histological findings of infiltration of abundant lymphocytes, plasma cells, and eosinophils. According to the report by Abraham *et al.* [25], moderate eosinophilic infiltration in the pancreas was observed histologically in five (21%) of 24 AIP patients, and these patients also had prominent eosinophilic infiltrates elsewhere in the biliary tract, but the eosinophilic infiltration was scattered within lymphoplasmacytic infiltration, which differed from the diffuse and dense eosinophilic infiltration seen in eosinophilic pancreatitis. They also reported that 10 (41%) of the 24 AIP patients had allergic and/or atopic manifestations, including asthma (n=8), allergic rhinitis (n=6), chronic urticaria (n=1), and nasal polyp (n=1), and in the 13 patients in whom a peripheral eosinophil count was performed, three showed mild eosinophilia (400–550 cells/mm^3) and one showed marked eosinophilia (1500 cells/mm^3) [25]. It has also been reported that patients with eosinophilic pancreatitis, a rare pancreatic condition usually showing systemic eosinophilic manifestations, have histories of allergies, asthma, or eczema [26,27].

This is the second report dealing with allergic manifestations in AIP patients following the study by Abraham *et al.* [25]. Interestingly, in this study, the occurrence of allergic-type AIP showed seasonality (September to February) that might be similar to that of other allergic diseases, such as bronchial asthma and acute allergic rhinitis [28,29]. This may also suggest a relationship between AIP and allergy. Although the reason for the difference is unknown, obstructive jaundice was a less frequent initial symptom in allergic-type AIP patients, and abdominal pain was detected in only five patients with allergic-type AIP.

The small sample size and the incomplete data set as a result of the retrospective nature of the study are limitations of this study. However, if some AIP cases are caused with allergic mechanisms, a new therapeutic strategy, such as antiallergic treatment, may be effective in these patients.

In conclusion, allergic manifestations were detected in about half of the AIP patients, and allergic mechanisms may be related to the occurrence of AIP in these patients.

Acknowledgement

Conflicts of interest: none declared.

References

1 Okazaki K, Chiba T. Autoimmune related pancreatitis. *Gut* 2002; 51:1–4.
2 Kim KP, Kim MH, Song MH, Lee SS, Seo DW, Lee SK. Autoimmune chronic pancreatitis. *Am J Gastroenterol* 2004; 99:1605–1616.
3 Finkelberg DL, Sahani D, Deshpande V, Brugge WR. Autoimmune pancreatitis. *N Engl J Med* 2006; 355:2670–2676.
4 Chari ST, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, Zhang L, *et al.* Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. *Clin Gastroenterol Hepatol* 2006; 4:1010–1016.
5 Kamisawa T, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroenterol* 2006; 41:613–625.
6 Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality: proposal of the concept of autoimmune pancreatitis. *Dig Dis Sci* 1995; 40:1561–1568.
7 Kamisawa T, Funata N, Hayashi Y, Eishi Y, Koike M, Tsuruta K, *et al.* A new clinicopathological entity of IgG4-related autoimmune disease. *J Gastroenterol* 2003; 38:982–984.
8 Kamisawa T, Nakajima H, Egawa N, Funata N, Tsuruta K, Okamoto A. IgG4-related sclerosing disease incorporating sclerosing pancreatitis, cholangitis, sialadenitis and retroperitoneal fibrosis with lymphadenopathy. *Pancreatology* 2006; 6:132–137.
9 Zen Y, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, *et al.* Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007; 45:1538–1546.
10 Robinson DS, Larche M, Durham SR. Tregs and allergic disease. *J Clin Invest* 2004; 114:1389–1397.
11 Otsuki M, Chung JB, Okazaki K, Kim MH, Kamisawa T, Kawa S, *et al.* Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea Symposium on Autoimmune Pancreatitis. *J Gastroenterol* 2008; 43:403–408.

12 Okazaki K, Kawa S, Kamisawa T, Naruse S, Tanaka S, Nishimori I, *et al.* Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. *J Gastroenterol* 2006; **41**:626–631.
13 Tefferi A. Blood eosinophilia: a new paradigm in disease classification, diagnosis, and treatment. *Mayo Clin Proc* 2005; **80**:75–83.
14 Waldram R, Kopelman H, Tsantoulas D, Williams R. Chronic pancreatitis, sclerosing cholangitis, and Sicca complex in two siblings. *Lancet* 1975; 1:550–552.
15 Muntefuso PP, Geiss AC, Bronzo RL, Randall S, Kahn E, McKinley MJ. Sclerosing cholangitis, chronic pancreatitis, and Sjogren's syndrome: a syndrome complex. *Am J Surg* 1984; **147**:822–826.
16 Kamisawa T, Egawa N, Tsuruta K, Okamoto A, Funata N. Primary sclerosing cholangitis may be overestimated in Japan. *J Gastroenterol* 2005; **40**:318–319.
17 Kamisawa T, Egawa N, Nakajima H, Tsuruta K, Okamoto A. Extrapancreatic lesions in autoimmune pancreatitis. *J Clin Gastroenterol* 2005; **39**:904–907.
18 Kamisawa T, Nakajima H, Hishima T. Close correlation between chronic sclerosing sialadenitis and immunoglobulin G4. *Intern Med J* 2006; **36**:527–529.
19 Hamano H, Kawa S, Horiuchi A, Unno H, Furuta N, Akamatsu T, *et al.* High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001; **344**:732–738.
20 Kamisawa T, Okamoto A, Funata N. Clinicopathological features of autoimmune pancreatitis in relation to elevation of serum IgG4. *Pancreas* 2005; **31**:28–31.
21 Aalberse RC, Milligen FV, Tan KY, Stapel SO. Allergen-specific IgG4 in atopic disease. *Allergy* 1993; **48**:559–569.
22 Nishimori I, Suda K, Oi I, Ogawa M. Autoimmune pancreatitis [in Japanese with English abstract]. *Nippon Shokakibyo Gakkai Zasshi* 2000; **97**:1355–1363.
23 Ito T, Nakano I, Koyanagi S, Miyahara T, Migita Y, Ogoshi K, *et al.* Autoimmune pancreatitis as a new clinical entity – three cases of autoimmune pancreatitis with effective steroid therapy. *Dig Dis Sci* 1997; **42**:1458–1468.
24 Sasahira N, Kawabe T, Nakamura A, Shimura K, Shimura H, Itobayashi E, *et al.* Inflammatory pseudotumor of the liver and peripheral eosinophilia in autoimmune pancreatitis. *World J Gastroenterol* 2005; **11**:922–925.
25 Abraham SC, Leach S, Yeo CJ, Cameron JL, Murakata LA, Boitnott JK, *et al.* Eosinophilic pancreatitis and increased eosinophils in the pancreas. *Am J Surg Pathol* 2003; **27**:334–342.
26 Flejou JF, Potet F, Bernades P. Eosinophilic pancreatitis: a rare manifestation of digestive allergy? *Gastroenterol Clin Biol* 1989; **13**:731–733.
27 Waguet J, Benamara MA, Chardot C, Pariente D. Common bile duct stenosis with hypereosinophilia and pancreatitis in childhood: report of 2 cases. *Pediatr Radiol* 2000; **30**:383–385.
28 Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann Allergy Asthma Immunol* 2003; **91**:288–296.
29 Okubo K, Gotoh M, Fujieda S, Okano M, Yoshida H, Morikawa H, *et al.* A randomized double-blind comparative study of immunotherapy for cedar pollinosis. *Allergology International* 2008; **57**:265–275.

Frequent and Significant K-*ras* Mutation in the Pancreas, the Bile Duct, and the Gallbladder in Autoimmune Pancreatitis

Terumi Kamisawa, MD, PhD, Kouji Tsuruta, MD, PhD,† Atsutake Okamoto, MD, PhD,‡ Shin-ichirou Horiguchi, MD,§ Yukiko Hayashi, PhD,§ Xiaoqing Yun, PhD,|| Toshikazu Yamaguchi, PhD,|| and Tsuneo Sasaki, MD, PhD¶*

Objectives: To assess the relationship between autoimmune pancreatitis (AIP) and pancreatic cancer, we analyzed K-*ras* mutation in the pancreatobiliary tissues of patients with AIP.

Methods: An analysis of K-*ras* mutation and an immunohistochemical study were performed on the pancreas of 8 patients with AIP and 10 patients with chronic alcoholic pancreatitis and on the common bile duct and the gallbladder of 9 patients with AIP. K-*ras* mutation was analyzed in the pure pancreatic juice from 3 patients with AIP.

Results: High-frequency K-*ras* mutation (2+ or 3+) was detected in the pancreas of all the 8 patients and in the pancreatic juice of the other 2 patients. The mutation in codon 12 of the *ras* gene was GAT in all the 10 patients. High-frequency K-*ras* mutation was detected in the common bile duct of 5 patients with AIP and in the gallbladder epithelium of 4 patients with AIP. The K-*ras* mutation was detected in the fibroinflammatory pancreas, the bile duct, and the gallbladder, with abundant infiltrating IgG4-positive plasma and Foxp3-positive cells of patients with AIP with elevated serum IgG4 levels.

Conclusions: Significant K-*ras* mutation occurs most frequently in the pancreatobiliary regions of patients with AIP. Autoimmune pancreatitis may be a risk factor of pancreatobiliary cancer.

Key Words: autoimmune pancreatitis, K-*ras*, pancreatic cancer, IgG4, Foxp3

(*Pancreas* 2009;38: 890–895)

Autoimmune pancreatitis (AIP) is a type of pancreatitis with presumed autoimmune etiology and is a relatively newly characterized disease entity, with much of our knowledge of it gained in only the last decade. However, it is increasingly being recognized as a bona fide disease. Autoimmune pancreatitis occurs most often in elderly males who experience obstructive jaundice as the most common initial symptom. Pancreatic exocrine and endocrine functions are sometimes impaired. Occasionally, there is an association with various extrapancreatic lesions. Radiologically, AIP is characterized by enlargement of the pancreas and irregular narrowing of the main pancreatic duct. Serologically, AIP is characterized by elevation of serum IgG4 levels. Histopathologically, dense infiltrations of CD4- or CD8-positive T lymphocytes and IgG4-positive plasma cells occur with fibrosis and obliterative phlebitis in the pancreas. Autoimmune pancreatitis responds well to steroid therapy, and both structural and functional changes in AIP recover promptly after steroid therapy. In contrast to ordinary chronic pancreatitis, AIP appears to be reversible.[1–3] Although the precise long-term outcome for AIP remains unclear, the prognosis of AIP is generally better than that of chronic pancreatitis.[2–4] However, several reports of AIP associated with pancreatic cancer occurring simultaneously or during follow-up have recently been described.[5–8]

Mutations in codon 12 of the K-*ras* gene have been found in more than 90% of pancreatic adenocarcinomas.[9,10] K-*ras* mutation is also detected in mucous cell hyperplasia in chronic pancreatitis.[11,12] K-*ras* mutation may occur during a relatively early stage in the multistep carcinogenesis process in the pancreas.[9,11,12] Chronic pancreatitis is characterized by irreversible morphologic changes and is a generally accepted risk factor for pancreatic cancer.[13] To assess the relationship between AIP and pancreatic cancer, we analyzed K-*ras* mutation in the pancreatobiliary tissues of 11 patients with AIP.

MATERIALS AND METHODS

Study Patients and Materials

Between 1989 and 2008, AIP was diagnosed in a total of 55 patients based on the Asian diagnostic criteria for AIP.[14] No patients developed pancreatobiliary cancer during the mean ± SD follow-up period of 42.8 ± 25.4 months.

K-*ras* analysis and/or an immunohistochemical study were performed on tissue from 11 patients with AIP (9 men and 2 women; age range, 61–79 years; mean age, 68.9 ± 6.3 years). Histories of heavy drinking and smoking were observed in 0 and 2 patients, respectively. The initial symptom was obstructive jaundice due to stenosis of the common bile duct in 10 patients and abdominal pain in 1 patient (Table 1).

Study materials consisted of 6 pancreatoduodenectomied pancreases, common bile ducts, and gallbladders; surgical biopsy specimen of 1 pancreas; 2 surgically resected common bile ducts and gallbladders; 1 autopsied pancreas, common bile duct, and gallbladder; and 10 surgically resected pancreases from patients with chronic alcoholic pancreatitis. K-*ras* mutation was analyzed in samples of pure pancreatic juice collected from the pancreatic duct during endoscopic retrograde cholangiopancreatography with injection of secretin in 3 patients with AIP and in 20 patients with chronic alcoholic pancreatitis other than the 10 resected cases.

The surgically resected tissues, the tissue biopsy specimens, and the autopsied tissues were fixed in 10% neutral buffered formalin. Each 1-cm wide tissue section was examined. Tissue blocks were routinely processed and embedded in paraffin. Serial sections were cut at a thickness of 3 μm. All sections were

From the Departments of *Internal Medicine, and †Surgery, Tokyo Metropolitan Komagome Hospital; ‡Tokyo Cancer Detection Center; §Department of Pathology, Tokyo Metropolitan Komagome Hospital, Tokyo; ||Division of Clinical Development of Biomedical Laboratories, Saitama; and ¶Department of Chemotherapy, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan.

Received for publication April 4, 2009; accepted July 7, 2009.

Reprints: Terumi Kamisawa, MD, PhD, Department of the Internal Medicine, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan (e-mail: kamisawa@cick.jp).

TABLE 1. Clinical and Radiological Findings in 11 Patients With AIP

Patient	Type	CBD Stenosis	Long-Term Outcome
1	Segmental (H)	Lower	Currently alive 8 yr after the operation
2	Segmental (H)	Lower	Died of pneumonia 1 yr after the operation
3	Diffuse	Lower	Currently alive 12 yr after the operation
4	Diffuse	Lower	Died of pulmonary cancer 10 yr after the operation
5	Diffuse	Upper	Unclear 3 yr after the operation
6	Segmental (H)	Lower	Died of hepatic failure 1 yr after the operation
7	Segmental (H)	Lower	Unclear 2 yr after the operation
8	Diffuse	Lower	Died of pulmonary cancer 11 mo after diagnosis
9	Segmental (H)	Lower	Unclear 3 yr after the operation
10	Diffuse	None	Unclear 4 yr after the diagnosis
11	Diffuse	Lower	Currently alive 11 yr after the diagnosis

H indicates pancreatic head; CBD, common bile duct.

stained with hematoxylin and eosin (H&E) and were examined immunohistochemically.

All subjects provided written informed consent. This study was approved by the relevant institutional review boards.

Immunohistochemical Study

Immunohistochemistry was performed, on average, on 2 representative sections from each case using antibodies against IgG4 (The Binding Site, Birmingham, United Kingdom), Ki-67 (clone MIB-1; Immunotech SA, Marseille, France), p53 (CM1; Novocastra Laboratories, Newcastle, United Kingdom), and Foxp3 (clone 22509; Abcam, Oxford, United Kingdom). All sections were stained with the avidin-biotin horseradish peroxidase method (Vectastain elite ABC kit; Vector, Burlingame, Calif). Additional staining procedures used have all been previously reported.[15,16] The degree of infiltrating IgG4-positive plasma cells was classified as 3+ (more than 30/high-power field [HPF]), 2+ (10-30/HPF), 1+ (5-10/HPF), +/− (1-4/HPF), and − (0/HPF).[17] The Ki-67 labeling index was determined by counting a minimum of 500 cells in the area representing the most homogenous region of positive cells.

K-*ras* Mutation Analysis

Paraffin blocks were prepared for DNA extraction. Two lesions from 2 slides of the pancreatic duct, the common bile duct, and the gallbladder mucosal epithelia were used for DNA extraction from tissue from each patient. The target lesions, including the abnormal epithelium, were microdissected using a 20-gauge needle, comparing the slide with H&E staining in the same position. The extracted DNA was diluted with 5 mL of Takara DEXPAT (for DNA extraction from paraffin-embedded tissue; Takara Biomedical Inc, Otsu, Japan). DNA was extracted from the pancreatic juice by the standard phenol/chloroform method.

Mutation of K-*ras* codon 12 was analyzed and compared by enriched polymerase chain reaction–enzyme-linked minisequence assay (PCR-ELMA).[18–20] In PCR-ELMA, the upstream primer for the first and second PCRs was 5′-TAAACTTGTGG TAGTTGGAACT-3′, the downstream primer for the first PCR was 5′-GTTGGATCATATTCGTCCAC-3′, and the downstream primer for the second PCR was 5′-CAAATGATCTGAAT TAGCTG-3′. The first PCR reaction was performed using 1 μL of DNA lysate, 100 μmol/L of deoxyribonucleotide triphosphates, 1.5 mmol/L of $MgCl_2$, 1 μmol/L of each primer, 0.625 U of Taq DNA polymerase (Perkin Elmer, Norwalk, Conn), and 1× PCR buffer (containing 10 mmol/L of Tris-HCl [pH 8.3 at 25°C], 50 mmol/L of KCl, and 0.001% [wt/vol] gelatin) in a thermal cycler (Perkin Elmer PJ-2000). Polymerase chain reaction amplifications proceeded at 95°C for 2 minutes, followed by 25 cycles at 95°C for 40 seconds, 60°C for 40 seconds, and 72°C for 40 seconds and a final extraction step of 7 minutes at 72°C. One microliter each of 10-fold dilutions of the first PCR-amplified product (93 base pairs) was taken for digestions with 0.5 μL (2.5 U) of *Bsr*I (New England Biolabs, Inc, Ipswich, Mass), and the 3.5-μL reaction buffer was composed of 100 mmol/L of NaCl, 50 mmol/L of Tris-HCl, 10 mmol/L of $MgCl_2$, and 1 mmol/L of dithiothreitol at 65°C for more than 15 hours in a total of 5 μL. For digestion, 45 μL of the second-stage reaction mixtures containing 1 μmol/L (each) of primers F1 and R2, and other components as those in the first-stage reaction mixtures were added to the previously mentioned restriction endonuclease reaction tubes. Subsequently, the second PCR amplifications were performed for 40 cycles with the same thermal cycle condition as the first PCR. Then, 10 μL of the denatured second PCR product was hybridized with probes to detect the K-*ras* codon 12 wild type (GGT) 6 mutants (GAT, GCT, GTT, AGT, CGT, and TGT). DNAs were immobilized at 55°C for 30 minutes, 100 μL of biotinalated A and 0.01 U of Tdq DNA polymerase were added, and incubation was continued at 55°C for 30 minutes.

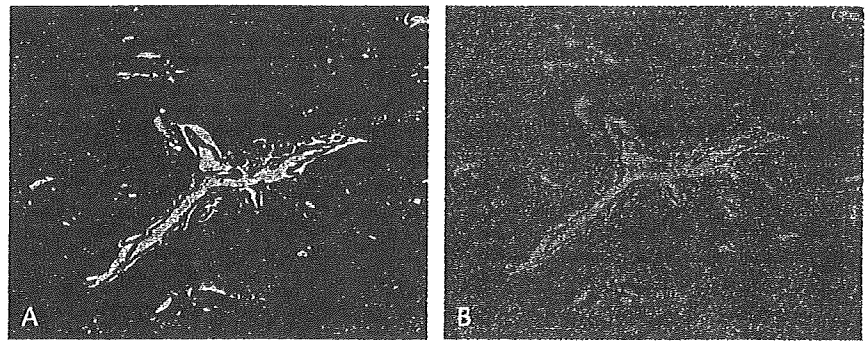

FIGURE 1. A, Histopathological findings in the pancreas with AIP showing periductal dense lymphoplasmacytic infiltration and fibrosis (H&E). The epithelium of the pancreatic duct was nearly well preserved with focal PanIN-1A. B, Abundant infiltration of IgG4-positive plasma cells in the periductal area (IgG4 immunostaining).

TABLE 2. Ki-67 Labeling Index and p53 Overexpression in the Pancreas, the Common Bile Duct, and the Gallbladder of 9 Patients With AIP

	Pancreas		Common Bile Duct		Gallbladder	
Patient	Ki-67, %	p53	Ki-67, %	p53	Ki-67, %	p53
1	1.8	—	1.1	—	1.5	—
2	3.2	—	1.2	—	0.8	—
3	0.9	—	2.1	—	0.6	—
4	2.5	—	1.1	—	0.7	—
5	1.2	—	1.2	—	0.8	—
6	1.0	—	1.7	—	0.7	—
7	2.1	—	2.9	—	2.1	—
8	3.3	—	1.3	—	0.5	—
9	Not examined		1.4	—		

To develop color, 100 μL of avidin-horseradish peroxidase conjugate was added, and the reaction was conducted at room temperature for 30 minutes. After a washing step, 100 μL of tetramethyl-benzidine substrate was added and the plates were left to develop in the dark at room temperature for 20 minutes. Finally, 100 μL of stop solution was decanted, and the light absorbance of each sample was measured by spectrophotometry (Multiskan Multisoft; Labsystems, Tokyo, Japan) with a 450-nm filter wavelength.

The amplified K-*ras* gene by PCR was captured to the probes that were designed to detect the K-*ras* codon 12 wild type (GGT) and 6 mutants (GAT, GTT, CGT, TGT, AGT, and GCT), which were finally measured using a microtiter plate reader for detection and quantification. The results of the semiquantitative analysis were scored as 3+, 2+, 1+, +/−, and − according to the percentage of mutant *ras* gene. Approximately, 3+, 2+, 1+, +/−, and − represented more than 20%, 2% to 20%, 0.2% to 2%, fewer than 0.2%, and none (not detected) of the mutants, respectively, according to the manufacturer.[19,20]

Statistical Analysis

The degree of infiltration of IgG4-positive plasma cells and the frequencies of K-*ras* mutation were scored as 3, 2, 1, 0.5, and 0, and they were analyzed with a Mann-Whitney *U* test. All reported *P* values are 2 sided. A $P < 0.05$ was considered significant.

RESULTS

Histopathological and Immunohistochemical Findings

Histopathologically, marked lymphoplasmacytic infiltration, periductal and interlobular fibroses, obliterative phlebitis in the pancreas (Fig. 1A), and transmural fibrosis with marked lymphoplasmacytic infiltration in the common bile duct were detected in the tissues from all patients with AIP. Transmural fibrosis with lymphoplasmacytic infiltration was detected in the gallbladder wall of 5 patients (patients 1–4 and 7). Pancreatic intraepithelial neoplasia (PanIN)-1A[21] (n = 6) and PanIN-1B (n = 2; patients 4 and 8) were focally detected. The pancreatic duct epithelium appeared almost normal in 2 patients (patients 3 and 6). There were no atypical changes in the epithelium of the common bile duct or the gallbladder in any patients. The mean (SD) Ki-67 labeling index was 2.0 ± 0.9% in the pancreatic duct, 1.5 ± 0.6% in the common bile duct, and 0.9 ± 0.5% in the gallbladder (Table 2). Dense infiltrations of IgG4-positive plasma cells (2+ or 3+) in the pancreas (Fig. 1B), the bile duct, and the gallbladder were detected in 100%, 89%, and 67% of patients, respectively (Table 3). Intensive fibroinflammation was detected in association with dense infiltration of IgG4-positive plasma cells. Foxp3-positive cells were distributed prominently in these fibroinflammatory lesions with many IgG4-positive plasma cells.

In chronic alcoholic pancreatitis, PanIN-1A and PanIN-1B were detected in all patients, and PanIN-2 was detected in 5 patients. The number of infiltrating IgG4-positive plasma cells was fewer than 5/HPF in the pancreas. The mean Ki-67 labeling index was 3.2 ± 0.9%. Overexpression of p53 was not observed in any specimen.

K-*ras* Mutation

High-frequency K-*ras* mutation (2+ or 3+) was detected in the pancreas of all 8 patients and in the pancreatic juice of the other 2 patients. The mutant type of *ras* gene was GAT in all the 10 patients (Table 3; Fig. 2A). In patients with chronic alcoholic pancreatitis, high-frequency K-*ras* mutation was detected in 4 (GAT, n = 2; TGT, n = 1; and GAT/GCT, n = 1)

TABLE 3. Serum IgG4 Levels, Distribution of IgG4-Positive Plasma Cells, and K-*ras* Mutations in the Pancreas, the Common Bile Duct, and the Gallbladder of 11 Patients With AIP

	Serum IgG4,	Pancreas		Pancreatic Juice,	Common Bile Duct		Gallbladder	
Patient	mg/dL	IgG4+ Cells	K-*ras*	K-*ras*	IgG4+ Cells	K-*ras*	IgG4+ Cells	K-*ras*
1	505	3+	3+ (GAT)	NE	3+	3+ (TGT)	3+	2+ (GAT)
2	550	3+	3+ (GAT)	NE	3+	3+ (GAT)	3+	3+ (GTT)
3	1240	3+	3+ (GAT)	NE	3+	3+ (GCT/TGT)	3+	3+ (GCT)
4	150	3+	3+ (GAT)	NE	3+	3+ (GAT)	2+	NA
5	43	3+	3+ (GAT)	NE	2+	NA	2+	+/− (GAT)
6	128	3+	3+ (GAT)	NE	2+	+/− (GCT)	1+	+/− (GAT)
7	780	3+	3+ (GAT)	1+ (GAT)	3+	3+ (GAT)	3+	3+ (TGT)
8	NE	3+	3+ (GAT)	NE	1+	1+ (TGT)	1+	NA
9	119	NE	NE	NE	2+	+/− (GCT)	1+	+/− (TGT)
10	NE	NE	NE	3+ (GAT)	NE	NE	NE	NE
11	298	NE	NE	2+ (GAT)	NE	NE	NE	NE

NE indicates not examined; NA, not amplified.

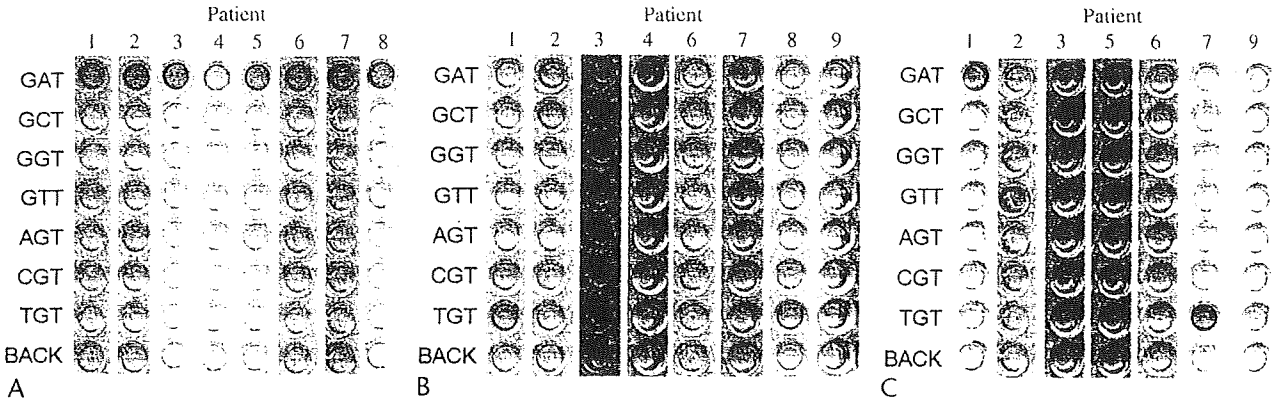

FIGURE 2. Actual images of microwell plates for semiquantitative analysis of mutant K-*ras* gene by PCR-ELMA in the pancreas (patients 1–8) (A), the common bile duct (patients 1–4 and 6–9) (B), and the gallbladder (patients 1–3, 5–7, and 9) (C). Hybridization was performed using both the wild type (GGT) and the 6 kinds of mutant-specific probes (GAT, GCT, GTT, AGT, CGT, and TGT) that were immobilized to the microtiter plate well. The type and semiquantity of the K-*ras* were identified as mutant type 3+ when the signal was observed exclusively in the mutant-specific probe well.

of the 10 resected pancreases (40%) and in 2 (GAT, n = 1; GAT/GGT/GTT, n = 1) out of 20 (10%) of the samples of pancreatic juice.

High-frequency K-*ras* mutation was detected in the common bile duct of 5 patients with AIP (GAT, n = 3; TGT, n = 1; and GCT/TGT, n = 1; Fig. 2B) and in the gallbladder epithelium of 4 patients with AIP (GAT, n = 1; TGT, n = 1; GCT, n = 1; and GTT, n = 1; Fig. 2C). The 4 patients with high-frequency K-*ras* mutation in the gallbladder also showed high-frequency K-*ras* mutation in the common bile duct, but the mutations in the *ras* gene were different. Low-frequency K-*ras* mutation (+ or +/−) was detected in the common bile duct of 3 patients and in the gallbladder of 3 patients (Table 3).

Relationship Between Serum IgG4 Levels and Infiltration of IgG4-Positive Plasma Cells and K-*ras* Mutation in the Common Bile Duct and the Gallbladder

Serum IgG4 levels were elevated in 6 patients (>135 mg/dL; Table 3). The scores for the infiltration of IgG4-positive plasma cells in the common bile duct (3,3,3,3,3) and in the gallbladder (3,3,3,3,2) of patients with AIP with high serum IgG4 levels were significantly higher than the scores in the common bile duct (2,2,2) and in the gallbladder (2,1,1) of patients with normal serum IgG4 levels ($P = 0.05$ and $P = 0.04$, respectively).

The scores for the K-*ras* mutation in the common bile duct (3,3,3,3,3) and in the gallbladder (3,3,3,2) of patients with AIP with high serum IgG4 levels were significantly higher than the scores in the common bile duct (0.5, 0.5) and in the gallbladder (0.5,0.5,0.5) of patients with normal serum IgG4 levels ($P = 0.02$ and $P = 0.04$, respectively).

DISCUSSION

This study revealed 3 important, new findings regarding AIP.

First, high-frequency K-*ras* mutation was detected in the pancreas of all 8 patients with AIP and in the pancreatic juice of the other 2 patients with AIP. The mutant type of *ras* gene was GAT in all the 10 patients. On the other hand, in the patients with chronic alcoholic pancreatitis, high-frequency K-*ras* mutations in the resected pancreas and the pancreatic juice were only detected in 40% and 10% of the samples, respectively.

K-*ras* mutation is believed to occur at a relatively early stage during the multistep carcinogenesis process. K-*ras* mutation was found in mucous cell hyperplasia in chronic pancreatitis, a condition that is considered to be a risk factor for the development of pancreatic cancer.[11–13] The incidence of K-*ras* mutation in chronic pancreatitis was reported to be 27%.[22] According to Tada et al,[23] K-*ras* mutation was detected in 19 (24%) of 79 cases of hyperplastic epithelium of the pancreas and in all 30 cases of pancreatic cancer. K-*ras* mutation was not detected in a normal pancreatic duct epithelium. Six mutant types of *ras* gene were detected in the hyperplastic epithelium of the pancreas including GAT (21%), GTT (21%), CGT (5%), TGT (37%), and AGT (16%), and the mutant types detected in pancreatic cancer were GAT (53%), GTT (33%), and CGT (14%).[23] The frequencies and types of K-*ras* mutation detected in the pancreas of patients with AIP were quite different from those of the hyperplastic epithelium.

The cumulative risk of pancreatic cancer in subjects with chronic pancreatitis was reported to be 1.8% after 10 years and 4.0% after 20 years.[13] Regarding AIP, 4 cases of pancreatic cancer associated with AIP have been reported in the English literature.[5–8] Of these 4 cases, pancreatic cancer was diagnosed simultaneously with AIP in 2 cases,[5,8] 1 case of pancreatic cancer developed 5 years after pancreatoduodenectomy for AIP[6] and the other developed 3 years after starting steroid therapy.[7] We have experienced no cases of pancreatic cancer associated with AIP, but an additional 3 cases were reported in the Japanese literature.[24–26] Localizations of these 7 pancreatic cancers were in the pancreatic head (n = 1), the body (n = 3), and the tail (n = 4). Recently, elevation of serum IgG4 levels was detected in 7.0%[27] to 9.6%[28] of patients with pancreatic cancer. Because AIP is a relatively rare and newly described type of pancreatitis, the prevalence of developing pancreatic cancer in AIP is currently unclear. However, in contrast to pancreatic cancer that typically occurs decades after the onset of chronic pancreatitis, pancreatic cancer associated with AIP developed during a much shorter time frame. This may be because K-*ras* mutation occurred far more frequently and significantly in the pancreatic ducts of patients with AIP compared with patients with chronic pancreatitis.

Our second significant finding is that high-frequency K-*ras* mutation was detected in the common bile duct epithelium of 5 patients with AIP and in the gallbladder epithelium of 4 patients with AIP. Four mutant types were detected in the biliary

tract, although only GAT was detected in the pancreas. The incidence rates of K-*ras* mutation in patients with bile duct cancer and patients with gallbladder cancer was reported to be 20%[29] to 100%[30] and 38%[31] to 55%,[30] respectively. The mutant type in the bile duct and the gallbladder cancers was reported to be GAT in 50%[32] to 80%[30] and 86%[30] of mutation-positive cases, respectively.

Sclerosing cholangitis is the most frequent extrapancreatic lesion seen in patients with AIP (40/55; 73% in our series). However, sclerosing cholangitis associated with AIP is quite different from primary sclerosing cholangitis, which sometimes develops into bile duct cancer, because it is responsive to steroid therapy and has abundant infiltrating IgG4-positive plasma cells in the intrahepatic bile duct wall in contrast to primary sclerosing cholangitis.[33,34] Sclerosing cholecystitis was found in 24% of our patients with AIP. It is recently suggested that sclerosing cholangitis and cholecystitis occur via the same mechanism as AIP (called as IgG4-related sclerosing disease) because the histopathological findings are similar and patients with either condition respond well to steroid therapy.[34,35] Recently, early bile duct cancer in a patient with sclerosing cholangitis with abundant infiltration of IgG4-positive plasma cells associated with AIP was histologically confirmed in the resected specimen.[36] Because high-frequency K-*ras* mutation was found in the biliary tract of half of the patients with AIP we studied, sclerosing cholangitis and sclerosing cholecystitis associated with AIP may be a risk factor for biliary cancer.

A third important observation from our study is that the degrees of K-*ras* mutation in the bile duct and the gallbladder of patients with AIP were correlated with the degree of fibroinflammation with infiltration of IgG4-positive plasma cells. Pancreatic intraepithelial neoplasias, ductal precursor lesions giving rise to invasive pancreatic adenocarcinoma, are classified from low grade (PanIN-1) to high grade (PanIN-3).[12,21] K-*ras* mutation is thought to be the initiating mutation responsible for PanIN-1 lesions.[12] In cancer-associated PanIN lesions, there was a stepwise increase in K-*ras* mutation that correlated with the grade of dysplasia.[37] However, abnormal changes in the pancreatic duct epithelium were less frequent in patients with AIP than in patients with chronic pancreatitis. The epithelia of the bile duct and the gallbladder of all 9 patients with AIP also showed no hyperplastic or dysplastic changes. Increased proliferation of cells and p53 overexpression were not detected in any specimen. K-*ras* mutation in patients with AIP may occur in a mechanism different from chronic pancreatitis.

Zen et al[38] reported that forkhead box P3 (Foxp3)–positive regulatory T cells, producing interleukin 10 and transforming growth factor β, which was followed by IgG4 class switching and fibroplasias, were increasingly detected in the pancreas and the biliary tract of patients with AIP. The present study also demonstrated increasing infiltration of Foxp3-positive cells in the fibroinflammatory lesions with many IgG4-positive plasma cells. On the other hand, Foxp3-positive regulatory T cells were increased locally in pancreatic cancer.[39] Furthermore, it was recently reported that Foxp3-positive regulatory T cells and inflammation played an essentially important role for K-*ras*-mediated lung tumorigenesis in mice.[40]

In our series, spontaneous improvement occurred in 3 patients with AIP, and 4 asymptomatic patients with AIP with segmental pancreatic enlargement have demonstrated no changes without steroid therapy during 1 to 2.5 years. Autoimmune pancreatitis is usually diagnosed at active stage, but IgG4-related fibroinflammation may have persisted subclinically in the pancreatobiliary regions for a long time. In patients with AIP, K-*ras* mutation may occur in the persistent fibroinflammatory pancreas and the biliary tract with Foxp3-positive cells.

This study is the first to reveal frequent and significant K-*ras* mutation in the pancreatobiliary regions of patients with AIP. The analyzed cases in this study are too small to be conclusive, but these findings suggest that AIP may be a risk factor of pancreatobiliary cancer. Further study of more cases and other sites of the pancreas including the mechanism of K-*ras* mutation should be necessary. However, because AIP may predispose patients to development of pancreatic or biliary cancer, diagnosis and follow-up of AIP should be conducted with careful attention to the possibility of pancreatobiliary cancer occurring simultaneously or within a short time after AIP diagnosis.

In conclusion, significant K-*ras* mutation was detected in the pancreas of all patients with AIP. The detection of K-*ras* mutation in the bile duct and the gallbladder was correlated with IgG4-related fibroinflammation with Foxp3-positive cells. Autoimmune pancreatitis may be a risk factor of pancreatobiliary cancer.

REFERENCES

1. Finkelberg DL, Sahani D, Deshpande V, et al. Autoimmune pancreatitis. *N Engl J Med.* 2006;355:2670–2676.
2. Okazaki K, Chiba T. Autoimmune related pancreatitis. *Gut.* 2002;51:1–4.
3. Gardner TB, Chari ST. Autoimmune pancreatitis. *Gastroenterol Clin N Am.* 2008;37:439–460.
4. Kamisawa T, Okamoto A. Prognosis of autoimmune pancreatitis. *J Gastroenterol.* 2007;42(suppl):59–62.
5. Inoue H, Miyatani H, Sawada Y, et al. A case of pancreas cancer with autoimmune pancreatitis. *Pancreas.* 2006;33:208–209.
6. Ghazale A, Chari S. Is autoimmune pancreatitis a risk factor for pancreatic cancer? *Pancreas.* 2007;35:376.
7. Fukui T, Mitsuyama T, Takaoka M, et al. Pancreatic cancer associated with autoimmune pancreatitis in remission. *Intern Med.* 2008;47: 151–155.
8. Witkiewicz AK, Kennedy EP, Kennyon L, et al. Synchronous autoimmune pancreatitis and infiltrating pancreatic ductal adenocarcinoma: case report and review of the literature. *Human Pathol.* 2008;79:630–634.
9. Almoguera C, Shibata D, Forrester K, et al. Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. *Cell.* 1988; 53:549–554.
10. Smit VTHB, Boot AJ, Smits AMM, et al. K-*ras* codon 12 mutations occur very frequently in pancreatic adenocarcinoma. *Nucleic Acids Res.* 1998;16:7773–7782.
11. Yanagisawa A, Ohtake K, Ohnishi K, et al. Frequent c-Ki-*ras* oncogene activation in mucous cell hyperplasia of pancreas suffering from chronic inflammation. *Cancer Res.* 1993;53:953–956.
12. Maitra A, Fukushima N, Takaori K, et al. Precursors to invasive pancreatic cancer. *Adv Anat Pathol.* 2005;12:81–91.
13. Lowenfels AB, Maisonneuve P, Cavallini G, et al. Pancreatitis and the risk of pancreatic cancer. *N Engl J Med.* 1993;328:1433–1437.
14. Otsuki M, Chung JB, Okazaki K, et al. Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea Symposium on Autoimmune Pancreatitis. *J Gastroenterol.* 2008;43:403–408.
15. Kamisawa T, Tu Y, Egawa N, et al. Ductal and acinar differentiation in pancreatic endocrine tumors. *Dig Dis Sci.* 2002;47:2254–2261.
16. Kamisawa T, Funata N, Hayashi Y, et al. Pathological changes in the non-carcinomatous epithelium of the gallbladder in patients with a relatively long common channel. *Gastrointest Endosc.* 2004;60:56–60.
17. Kamisawa T, Funata N, Hayashi Y, et al. A new clinicopathological entity of IgG4-related autoimmune pancreatitis. *J Gastroenterol.* 2003;38:282–284.
18. Tada M, Teratani T, Komatsu Y, et al. Quantitative analysis of *ras*