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Inflammatory Abdominal Aortic Aneurysm with Immunoglobulin Heavy Chain Gene Rearrangement

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Abstract：Inflammatory abdominal aortic aneurysm（IAA）is an aortic manifestation of IgG4-related disease. However, the pathogenesis of IgG4-related disease is obscure. Herein, we report the case of a 74-year-old Japanese man with IAA, associated with IgG4-positive plasma cell infiltration and oligoclonal bands of immunoglobulin heavy chain（IgH）gene rearrangement. The patient presented with a growth of pulsating abdominal mass. Computed tomography revealed an infrarenal type of abdominal aortic aneurysm and right common iliac arterial aneurysm. Histologically, the aneurysmal wall of the aorta showed marked fibrous thickening of tunica adventitia with severe lymphoplasmacytic infiltration, including lymphoid follicles, perineural inflammatory cell infiltration, and obstructive phlebitis. Immunohistochemical analysis showed the infiltration of many IgG4-positive plasma cells and formation of B-cell rich lymphoid follicles in the aneurysmal wall. The mean percentage value of IgG4-positive plasma cells was found to be 64.9 ± 14% of all IgG-positive plasma cells. Light chain immunoglobulin restriction was absent. Polymerase chain reaction analysis showed oligoclonal bands of IgH gene rearrangement in all samples obtained from the aneurysmal wall. Thus, the present paper describes for the first time an IAA case with extensive infiltration of numerous IgG4-positive plasma cells, as well as oligoclonal bands of IgH gene rearrangement.

Key words：**Inflammatory abdominal aortic aneurysm, IgG4-positive plasma cell, IgH gene rearrangement, Polymerase chain reaction, Immunohistochemistrys**

Introduction

Inflammatory abdominal aortic aneurysm（IAA），first described by Walker in 1972,[1)] is characterized by a diffuse thickness of the aneurysmal wall, with extensive fibrosis and abundant infiltration of lymphocytes and plasma cells. Many hypotheses pertaining to the pathogenesis of IAA have been postulated, including the extreme end of an inflammatory reaction to atherosclerotic plaque substances（e.g., oxidized low-density lipoprotein and ceroid），[2)] systemic autoimmune diseases,[3)] and an exaggerated immune reaction to infectious agents.[4)] The newly defined disease entity "immunoglobulin G4-related disease"（IgG4RD）has been recently proposed with regard to autoimmune pancreatitis, and is pathologically characterized by diffuse infiltration of lymphocytes and plasma cells, extensive fibrosis, and obstructive phlebitis, irrespective of the affected organs[5, 6)]. We previously reported that IAA showed a significant increase in the number of infiltrating IgG4-positive plasma cells and a disrupted follicular dendritic cell network in lymph follicles, in comparison with ordinary atherosclerotic abdominal aortic aneurysm, suggesting that IAA may be an aortic lesion of IgG4RD[7)].

IgG4RD is considered a clinicopathologic disease entity that includes sclerosing cholangitis, autoimmune pancreatitis, Mikulicz's disease, Sjogren's syndrome, and tubulointerstitial nephritis[6)]. IAA and

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idiopathic retroperitoneal fibrosis have been reported to belong to IgG4RD[7, 8, 9]. Recently, idiopathic retroperitoneal fibrosis has demonstrated monoclonal or oligoclonal immunoglobulin heavy chain (IgH) gene rearrangement[10]. In addition, there has been a report of several cases with ocular adnexal mucosa-associated lymphoid tissue lymphoma arising from IgG4RD, occurring in the same organ[11]. These cases displayed immunoglobulin light chain restriction and IgH gene rearrangement. However, another report demonstrated an absence of IgH gene rearrangement in cases with IgG4-related retroperitoneal and mediastinal fibrosis and idiopathic retroperitoneal fibrosis[12, 13]. Additionally, little is known about IgH gene rearrangement in IAA.

We describe herein a case of IAA with infiltration of numerous IgG4-positive plasma cells and oligoclonal bands of IgH gene rearrangement. We also discuss the mechanisms potentially responsible for IgG4RD.

Clinical Summary

A 74 year-old man, with a history of a pulsating abdominal mass 2 years earlier, was referred to our hospital because of increased growth of the abdominal mass. He had angina pectoris 5 years earlier and undergone percutaneous coronary intervention. Upon presentation to our hospital, he had neither abdominal nor back pain. He had no history of diabetes mellitus, hypertension, dyslipidemia, obesity, renal disease, and autoimmune diseases, but he had a history of smoking (20 cigarettes per day for 50 years). Upon admission, his heart rate was regular at 60 beats/min, and his blood pressure was 145/75 mm Hg. The pulsating abdominal mass was located in the umbilical region. On laboratory examination, serum C-reactive protein was 0.1 mg/dL (reference range, 0.0–0.2); total protein, 7.6 g/dL (6.7–8.3); albumin, 4.0 g/dL (4.0–5.0); total cholesterol, 217 mg/dL (128–219); high-density lipoprotein cholesterol, 35 mg/dL (40–96); low-density lipoprotein cholesterol, 170 mg/dL (<139); blood urea nitrogen (BUN), 25 mg/dL (8–22); and creatinine (Cr), 1.4 (0.6–1.1) mg/dL. There were no data of serologic tests including IgG, IgG4, IgM, IgE, IgA, and antinuclear antibodies. A computed tomography showed an infrarenal type of abdominal aortic aneurysm (8 cm maximum diameter) and right common iliac arterial aneurysm (4 cm maximum diameter). We performed surgery with graft replacement of abdominal aorta (GORETEX 16X8X8) to intervene the abdominal aortic aneurysm.

Materials and Methods

A pathologic specimen from the abdominal aortic aneurysm was fixed in 10% formalin and routinely processed for light microscopic and immunohistochemical examination. Paraffin-embedded serial sections were prepared and stained with hematoxylin–eosin, elastica–van Gieson, Masson–Trichrome, and Gram and periodic acid-Schiff (PAS) stains for light microscopic observation. Some sections were examined immunohistochemically with an indirect method, using the primary antibodies listed in Table 1. DNA was extracted from paraffin-embedded tissue sections (3 × 5 mm) by overnight digestion with proteinase K and used for analysis of IgH gene rearrangement. After the quality of the DNA sample was confirmed by successful β-globin gene amplification, the samples were subjected to multiplex polymerase chain reaction (PCR) for detection of IgH gene rearrangement, such as VH FR1-JH, FR2-JH, FR3-

Table 1. Antibodies and staining methods used for immunohistochemistry

Antibody	Clone	Source	Dilution	Antigen retrieval method
IgG	polyclonal	SIGNET	1:4	Microwave heating
IgG4	HP6025	CHEMICON	1:100	Pronase treatment
λ	polyclonal	SIGNET	1:10000	Microwave heating
κ	polyclonal	SIGNET	1:10000	Microwave heating
CD20	L26	DAKO	1:100	Microwave heating
CD3	PS1	NOVO	1:100	Microwave heating

CHEMICON, Massachusetts, USA; DAKO, Glostrup, Denmark; NOVO, Hessen, Deutsch; SIGNET, New Jersey, USA.

JH, and DH-JH, according to BIOMED-2 protocols[14]. The PCR products were analyzed by heteroduplex polyacrylamide gel electrophoresis. A B-cell lymphoma sample was used as the positive control.

All tissue specimens were taken in compliance with a protocol stipulated by the Fukuoka University School of Medicine Human Research Subject Committee (No. 381).

Results

Pathologic findings

The surgical specimen showed a thickened aneurysmal wall of the abdominal aorta, which was approximately 8 mm in thickness. Advanced atherosclerosis was observed in the wall. As shown in Figures 1A and 1B, the aneurysmal wall showed marked fibrous thickening of tunica adventitia with lymphoid follicle formation. Tunica media showed atrophy with disruption and fragmentation of elastin fibers. Complicated plaque with thrombosis and ulceration was found in the aneurysmal wall. Diffuse and extensive lymphoplasmacytic infiltration was observed in the thickened adventitia (Figure 1C). Lymphoid follicles displayed a prominent germinal center (Figure 1D). Obstructive phlebitis and perineural inflammatory cell infiltration were also found in the adventitia (Figures 1E and 1F). There was no evidence of bacterial or fungal infection with Gram and PAS staining, respectively. Immunohistochemical staining showed the infiltration of many IgG4- and/or IgG-positive plasma cells in the aneurysmal wall (Figures 2A and 2B). IgG- and IgG4-positive plasma cells were counted in 10 different high-power fields of severe inflammatory areas in the wall, and the mean of the top five values of percentage of IgG4-positive cells to total IgG-positive cells was 64.9 ± 14%. Light chain immunoglobulin showed approximately equal proportions of κ and λ light chains in the lesion (Figures 2C and 2D). CD20-positive B cells were found exclusively in lymphoid follicles, whereas CD3-positive T

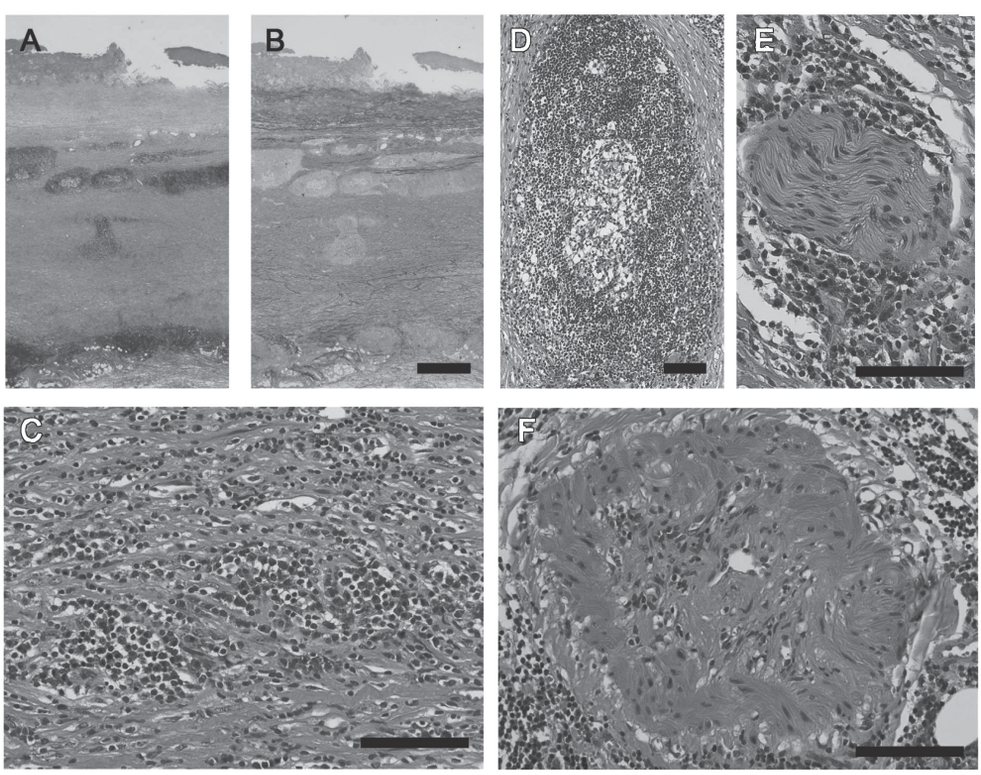

Figure 1. Histopathologic characteristics of the wall of the inflammatory abdominal aortic aneurysm. Low-magnification scanning views of the aneurysmal wall show fibroatheroma in the intima and thickened fibrotic adventitia with many lymph follicles (A, B). The tunica media shows marked atrophy and fragmentation of elastin fibers (B, arrow). High-magnification views of the aneurysmal wall show the diffuse and extensive infiltration of lymphocytes and plasma cells (C), lymph follicles with prominent germinal centers (D), perineural inflammatory cell infiltration (E), and obstructive phlebitis (F). A, C–F: hematoxylin-eosin stain; B: elastica–van Gieson stain; Bar = 1 mm (B), 0.1 mm (C–F).

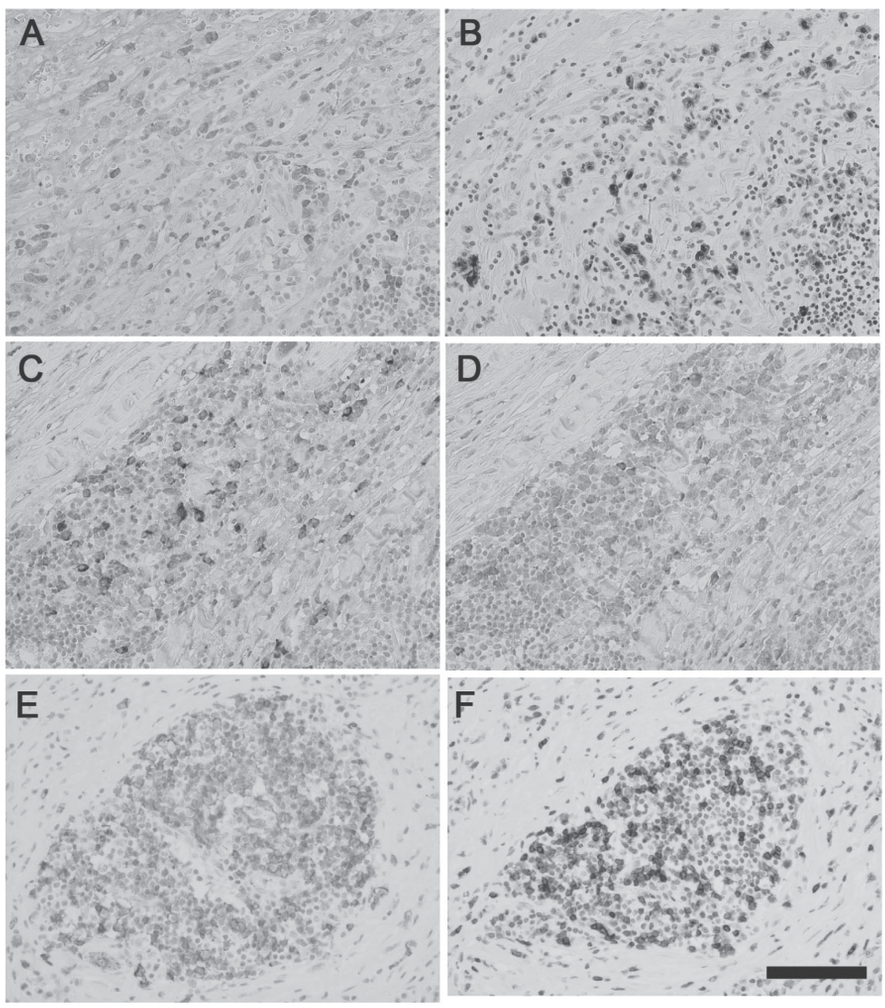

Figure 2. Immunohistochemical observation of the wall of the inflammatory abdominal aortic aneurysm. Note the diffuse infiltration of IgG- and/or IgG4-positive plasma calls in the wall (A, B). Plasma cells express k- and l- light chain immunoglobulins (C, D). CD20-positive B cells are found exclusively in lymphoid follicles, whereas CD3-positive T cells are distributed in lymphoid follicles and the surrounding tissue areas (E, F). A: IgG; B: IgG4; C: l; D: k ; E: CD20; F: CD3. Bar = 0.1 mm.

cells were distributed both in the lymphoid follicles and the surrounding areas (Figures 2E and 2F). Hence, the diagnosis of IgG4-related IAA was made.

IgH gene rearrangement analysis

We examined 4 different tissue samples of the aneurysmal wall for IgH gene rearrangement by using PCR. The results demonstrated oligoclonal bands of IgH gene rearrangement, including VH FR1-JH, VH FR2-JH, VH FR3-JH, and DH-JH gene rearrangements, which were detected in 4/4 (100%), 3/4 (75%), 3/4 (75%), and 3/4 (75%) tissues samples, respectively (Figure 3). The number of clonal bands was different among the various samples.

Discussion

The present study describes a case of IgG4-related IAA with the following characteristics: 1) marked fibrous thickening of the aneurysmal wall; 2) severe lymphoplasmacytic infiltration with lymphoid follicles, perineural inflammatory cell infiltration, and obstructive phlebitis; 3) diffuse and extensive infiltration of IgG4-positive plasma cells (IgG4+/IgG+ cell ratio, 64.9 ± 14%) ; and 4) oligoclonal bands of IgH gene rearrangements.

IAA is a clinicopathologic entity, distinct from an ordinary atherosclerotic aneurysm of the abdominal aorta[9]. Patients with IAA often have clinical manifestations, including fever, dull pain, and weight loss. Laboratory

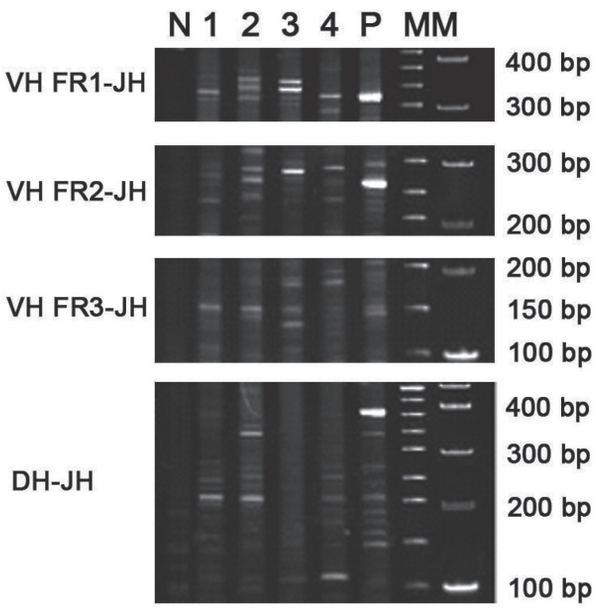

Figure 3. Polymerase chain reaction (PCR) analysis of immunoglobulin heavy chain (IgH) gene rearrangement. Monoclonal and oligoclonal bands of IgH gene rearrangement were found in all tissue samples obtained from different sites of the aneurysmal wall. VH-JH PCR was separately carried out using 3 VH primers corresponding to VH FR regions (FR1, FR2, and FR3) together with the consensus JH primer in an individual PCR. DH-JH PCR was performed using 6 family-specific DH primers together with the consensus JH primer. The expected PCR product sizes are as follows: VH FR1-JH: 310–360 base pair (bp); VH FR2-JH: 250–295 bp; VH FR3-JH: 100–170 bp; DH-JH: 110–290 bp and 390–420 bp. Lanes 1–4: four different samples in the present case; N: negative control; P: positive control; MM: molecular markers, including 50 and 100 bp ladders.

examinations show an inflammatory reaction, such as high levels of erythrocyte sedimentation rate and C-reactive protein. Radiologically, IAA is detected as a dilatation of the aorta with "mantle sign" by computed tomography. Histopathologically, IAA lesions are characterized by a thick fibrous aneurysmal wall with chronic inflammatory cell infiltration[1, 2]. The patient in the present case was asymptomatic, and showed aneurysmal dilatation of infrarenal abdominal aorta and right common iliac artery on imaging. Laboratory results showed mild elevation of BUN and Cr. Pathologic examination showed the marked fibrous thickening of the aneurysmal wall, 8 mm in thickness, and severe infiltration of lymphocytes and plasma cells with lymphoid follicles in the lesion. Thus, the present case demonstrated few clinical characteristics of IAA, but showed histopathologic characteristics that are consistent with IAA[1, 2, 4, 7].

IgG4RD is considered a systemic sclerosing disease that can involve the pancreas, bile duct, gallbladder, salivary gland, retroperitoneum, kidney, lung, and aorta[6]. IAA has been shown to be an aortic manifestation of IgG4RD[7]. Recently, it has been proposed that IAA can be subclassified into IgG4-related IAA and non-IgG4-related IAA[9]. Histopathologically, IgG4-related IAA is characterized by diffuse and extensive infiltration of IgG4-positive plasma cells, lymphoid follicle formation, perineural inflammatory cell infiltration, and obstructive phlebitis, all of which were detected in the present case. Moreover, the ratio of IgG4-positive cells to total IgG-positive cells was 64.9 ± 14%. Thus, our case may belong to an IgG4-related IAA.

An increasing number of studies[15, 16] support the role of immune reactions in the mechanism underlying IgG4RD. It was recently reported that the expression of Th2 cytokines and regulatory cytokines is up-regulated in organs affected by IgG4RD[17]. However, the pathogenesis of IgG4RD is still obscure. IAA and idiopathic retroperitoneal fibrosis are known to be included in the spectrum of "chronic periaortitis," characterized by a fibro-inflammatory reaction extending from the adventitia of the abdominal aorta into the retroperitoneum[9, 16]. Recently, IgG4RD was reported to include IAA as well as idiopathic retroperitoneal fibrosis [8, 12]. Idiopathic retroperitoneal fibrosis has been shown to demonstrate either monoclonal or oligoclonal IgH gene rearrangement, suggesting the possibility that this disease may have a potential predisposition to B-cell lymphoma[10]. Ocular adnexal mucosa-associated lymphoid tissue (MALT) lymphoma arising from IgG4RD has been shown to occur in the same organ and demonstrate IgH gene rearrangement and light chain restriction[18]. However, little is known about IgH gene rearrangement in IgG4-related IAA. In the present case, oligoclonal bands of IgH gene arrangement were detected in all samples from the aneurysmal wall. Immunohistochemical analysis showed the formation of B-cell-rich lymphoid follicles and the absence of light chain restriction in the lesion. These results suggest that oligoclonal, but not monoclonal, proliferation of reactive B cells may be related to the pathogenesis of IgG4-related IAA, which may have the risk of overt malignant lymphoma despite the absence of both histological and

clinical evidence of malignant lymphoma. Clearly, the lymphomagenesis in the context of IgG4RD requires larger studies involving more patients.

In summary, we describe here for the first time a case of IAA characterized by an extensive infiltration of numerous IgG4-positive plasma cells, as well as oligoclonal bands of IgH gene rearrangement. We also discussed a potential mechanism for the development of IgG4RD.

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