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【 CASE REPORT 】

Concurrent Autoimmune Neutropenia and Idiopathic Thrombocytopenic Purpura Associated with IgG4-related Diease

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Abstract:
IgG4-related disease (IgG4RD) is a multi-organ disorder characterized by an elevated serum IgG4 level and IgG4-positive plasma cell infiltration of the affected organs, accompanied by tissue fibrosis and sclerosis. Although it can affect any organ, to our knowledge, no cases involving concurrent autoimmune neutropenia and thrombocytopenia have been reported. A 62-year-old man visited our hospital and was diagnosed with IgG4RD accompanied by interstitial pneumonitis, lymphadenopathy, and interstitial nephritis. During his clinical course, he developed autoimmune neutropenia and idiopathic thrombocytopenic purpura. Our case, invoving multiple hematological abnormalities, might help deepen our understanding of the pathophysiology of IgG4RD.

Key words: IgG4-related disease, autoimmune neutropenia, idiopathic thrombocytopenic purpura

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Introduction

IgG4-related disease (IgG4RD) is a multi-organ immune-mediated disease (1). An elevated serum IgG4 level and tissue infiltration of the affected organs by IgG4-positive plasma cells are characteristics of IgG4RD (2). The first report of a high serum IgG4 level involved a patient with autoimmune pancreatitis (3). Once IgG4RD was recognized as a discrete entity, it was subsequently shown that it can affect multiple organs (1, 2). However, it often mimics malignancies, sarcoidosis, Castleman's disease, Sjögren's syndrome, and other conditions (1, 2). IgG4RD should therefore be diagnosed based on the current diagnostic criteria (4).

Although it is rare, concurrent idiopathic thrombocytopenic purpura (ITP) has been reported to occur as a hematological complication of IgG4RD (5). However, no cases involving simultaneous ITP, autoimmune neutropenia (AIN), and IgG4RD have been reported. The clinical and histological features of IgG4RD have gradually been clarified, but its pathophysiological features remain to be fully elucidated.

We herein report a case in which ITP and AIN arose in a patient with IgG4RD and discuss a possible cause of this unique complication from an immunological perspective.

Case Report

A 63-year-old Japanese man with no remarkable medical history was admitted to our hospital due to a persistent dry cough and abnormalities on a chest roentgenogram. He first visited the Department of Respiratory Medicine and underwent an ultrasound-guided lung biopsy, which was not diagnostic. Computed tomography (CT) showed swelling of the

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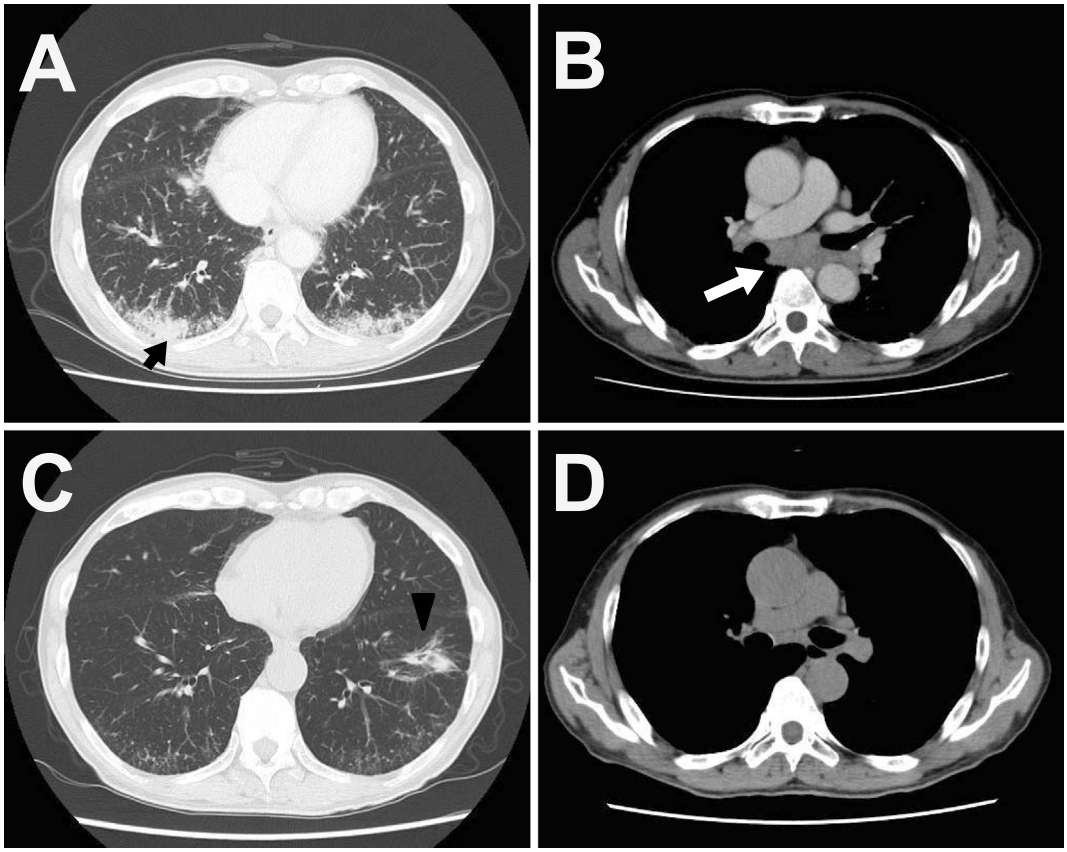

Figure 1. **Computed tomography of the chest before (A and B, contrast-enhanced) and after (C and D, plain) the therapy. Consolidation was remarkable along the sub-pleural area in the bilateral lower lobes. A biopsy was performed for the solid tumor-like lesion (black arrow) (A). Swelling of the lymph nodes around the bronchial bifurcation is depicted (white arrow) (B). The opaque areas of sub-pleural and lung fields showed marked improvement, except for the scars from the previous bout of bacterial pneumonia in the left lower lobe (arrowhead) (C). The swollen lymph nodes in the area were also markedly improved along with those of other parts of the body (D).**

mediastinal, para-aortic, and common iliac lymph nodes in addition to consolidation in the bilateral lower pulmonary lobes (Fig. 1A and B). To exclude the possibility of malignant lymphoma, he was referred to the Department of Hematology.

On admission, his height was 169 cm, and his weight was 65 kg (he had lost 3 kg within a year). A physical examination revealed fine crackles in both lungs. His peripheral blood exhibited mild thrombocytopenia and neutropenia associated with eosinophilia (Table 1). Blood chemistry tests demonstrated mildly elevated serum creatinine and blood urea nitrogen levels. The results of blood coagulation tests were within the normal ranges. The patient's serum IgE, IgG, and IgG4 levels were elevated to 1,560 IU/mL (normal range: <173 IU/mL), 5,489 mg/dL (normal range: 870-1,700 mg/dL), and 2,660 mg/dL (normal range: 4.9-105 mg/dL), respectively. His complement levels were low, contrary to his elevated immune complex level. Except for marginal positivity for the anti-nuclear antibody, tests for representative autoantibodies produced negative results. A urinalysis detected proteinuria (0.74 g/day) and microscopic hematuria along with scattered erythrocytes and epithelial casts. Urine chemistry tests revealed elevated β_2-microglobulin and N-acetyl-β-D-glucosaminidase. Bone marrow aspiration showed a normocellular marrow with eosinophilic hyperplasia and an increase in the number of immature megakaryocytes without any abnormal or dysplastic cells (nuclear cell count: 104,000/μL, myeloid/erythroid ratio: 3.3, megakaryocytes: 56/μL, myeloid cells: 60.0%, eosinophils: 15.2%). A G-banding analysis of the bone marrow cells revealed a normal male karyotype. The mild neutropenia and thrombocytopenia resolved without treatment within a few days. A second needle biopsy of the lung was performed from the basal part of the right lower lobe (Fig. 1A, black arrow). The lung specimen revealed an increased amount of fibro-connective tissue and invasion by plasma cells, small lymphocytes, and eosinophils (Fig. 2A). Immunostaining demonstrated an increased number of IgG4-positive plasma cells (Fig. 2B). An examination of the renal tissue revealed interstitial nephritis and the infiltration of IgG4-positive plasma cells, small lymphocytes, and eosinophils (Fig. 2C and D). The patient's clinical features and laboratory data, his elevated IgG4 (> 135 mg/dL) level, and the detection of an increased IgG4+ cell/IgG+ cell ratio (>40%) on a biopsy examination were

Table 1. The Representative Data on Admission.

CBC		Serum Immunology	
WBC	4,300 /μL	IgG	5,151 mg/dL
Seg	3 %	IgG4	2,660 mg/dL
Band	9 %	IgA	166 mg/dL
Eosin	52 %	IgM	66 mg/dL
Baso	2 %	IgE	1,560 IU/mL
Mono	11 %	C3	19 mg/dL
Lymph	23 %	C4	3 mg/dL
RBC	436 $\times 10^4$/μL	CH50	<12.0 CH50/mL
Hb	13.6 g/dL	C1q	26.4 μg/mL
Hct	38.5 %	ANA	(+)
PLT	6.9 $\times 10^4$/μL	Homo	×40
IPF*	4.9 %	Speckled	×40
		Anti-SS-A antibody	(-)
Biochemistry		Anti-SS-B antibody	(-)
TP	9.6 g/dL	Anti-Scl70 antibody	(-)
Alb	2.9 g/dL	Anti-Jo-1 antibody	(-)
AST	21 U/L	Anti-centromere antibody	(-)
ALT	17 U/L	Anti-Sm antibody	(-)
γ-GTP	22 U/L	Anti-RNP antibody	(-)
ALP	181 U/L	Anti-MPO-ANCA antibody	(-)
LDH	228 U/L	Anti-PR-3-ANCA antibody	(-)
T-Bil	1.0 mg/dL		
BUN	22.2 mg/dL	Urine Biochemistry	
CRE	1.37 mg/dL	U-TP	740 mg/day
β2MG	6.1 mg/L	U-NAG	14.6 U/L
CRP	0.2 mg/dL	U-β2MG	28,800 μg/L

IPF: immature platelet fraction, β2MG: β2-microglobulin, NAG: N-acetyl-β-D-glucosaminidase

compatible with the current diagnostic criteria for definite IgG4RD (4). Based on these results, he was diagnosed with IgG4RD associated with interstitial pneumonitis, lymphadenopathy, and interstitial nephritis.

Although we tried to start steroid therapy soon after the diagnosis, the patient contracted influenza A and then developed *Staphylococcus aureus* pneumonia. His pneumonia was treated with intravenous antibiotics for a month, but it was not completely cured. We were obliged to administer additional antibiotics orally for another month. During the treatment, the patient's neutrophil count decreased from 6,630 to 200/μL in a month, and his platelet count fell from 15.8× 10^4/μL to 1.6×10^4/μL within 2 weeks. The immature platelet fraction (IPF) conversely exhibited a remarkable increase from 1.4% to 18.1% (normal range: 1.9-4.8%). Although we changed his oral antibiotics in order to exclude drug-induced bicytopenia, it did not improve his bicytopenia. We therefore considered that autoimmune mechanisms associated with IgG4RD might be responsible for his bicytopenia.

The indirect granulocyte immunofluorescence test (GIFT) was used to detect anti-neutrophil antibodies. As a result, the patient's serum reacted with both human neutrophil antigen (HNA)-1a-homozygous neutrophils and HNA-1b-homozygous neutrophils (Table 2). ITP was diagnosed by the exclusion of other diseases that could cause thrombocytopenia, such as aplastic anemia, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, and other diseases, according to the guidelines of the Ministry of Health, Labor and Welfare (MHLF) Japan.

Although the patient had not made a full recovery from his *Staphylococcus aureus* pneumonia, we started administering steroids at a dose of 0.5 mg/kg/day. As shown in Fig. 3, his platelet and neutrophil counts recovered promptly to normal levels after the initiation of the treatment, and his serum IgG4 level showed a decreasing trend. His respiratory symptoms gradually improved along with the disappearance of his proteinuria and the normalization of his serum creatinine level. One month later, CT revealed marked improvement in the abnormalities of the lung and the swelling of the lymph nodes (Fig. 1C and D).

Discussion

As IgG4RD affects multiple organs simultaneously, systemic investigations are indispensable. Although a relationship has been suggested to exist between IgG4RD and ITP (5-12), only one case report discussing AIN as a complication of autoimmune pancreatitis has been reported thus far (13). Blood cytopenia occurs much less frequently as a complication of IgG4RD than other typical organ involvements. However, autoimmune diseases, infectious diseases, tumors, transplantations, and drugs often induce the develop-

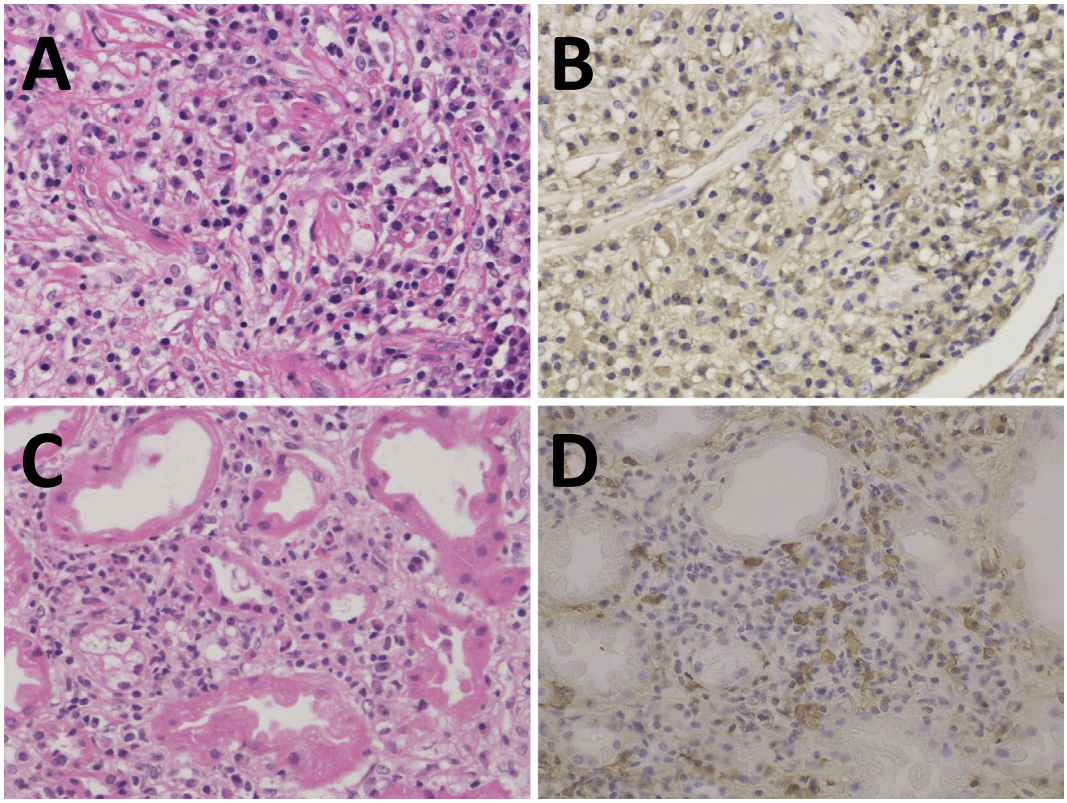

Figure 2. **Finding from a histological examination. A histological examination of the lung tissue that had been subjected to Hematoxylin and Eosin (H&E) staining showed lymphoplasmacytic infiltration together with abundant fibrosis (×40) (A). Immunostaining for IgG4 showed many IgG4-positive plasma cells (IgG4+/IgG+plasma cell ratio=50.4%, ×40) (B). A histological examination of the kidney tissue that had been subjected to H&E staining showed interstitial nephritis combined with lymphoplasmacytic infiltration (×40) (C). Immunostaining for IgG4 showed many IgG4-positive plasma cells (IgG4+/IgG+plasma cell ratio=83.0%, ×40) (D).**

Table 2. **Changes in the Titer of Anti-neutrophil Antibodies before and during the Steroid Therapy.**

Before Treatment			
	MFI		
Phenotype of panel neutrophils	Control	Patient	
HNA1a/1a	1,090	16,163	Positive*
HNA1b/1b	223	6,258	Positive

During Treatment			
	MFI		
Phenotype of panel neutrophils	Control	Patient	
HNA1a/1a	562	1,203	Positive
HNA1b/1b	787	1,318	Negative

GIFT: indirect granulocyte immunofluorescence test, MFI: mean fluorescence intensity, HNA: human neutrophil antigen

* Positive>MFI×2

ment of AIN, ITP and hemolytic anemia simultaneously (14). In most of these cases, therapy for the associated cytopenia is used to treat the underlying disease. In our case, AIN and ITP responded well to steroid therapy along with the patient's IgG4RD-related symptoms and signs. We therefore suspect that an association with AIN and ITP may be included among the clinical features of IgG4RD.

The presence of anti-neutrophil antibodies is essential for a diagnosis of AIN. In adults, AIN often occurs as a secondary condition as described above (15). Two months after the initiation of steroid therapy, the fluorescence evoked between the patient's serum and HNA-1a-homozygous neutrophils had diminished, and that between HNA-1b-homozygous neutrophils had disappeared (Table 2). We therefore tested him for autoantibodies against neutrophils as well as changes in the titer of such antibodies. Although we were unable to perform tests for autoantibodies against platelet surface proteins, such as platelet glycoprotein IIb/IIIa complex, ITP is usually diagnosed based on the exclusion of other conditions. During the patient's clinical course, his platelet counts and their IPF values demonstrated a converse association, indicating both accelerated platelet destruction and activated thrombopoiesis in his thrombocytopenic phase. The observed increase in the number of megakaryocytes in the bone marrow supported this notion. Circumstantial evidence consistently indicated that the patient's disease was complicated by ITP according to the guidelines outlined by MHLW Japan.

The IgG4 molecule undergoes Fab-arm exchange due to the instability of the disulfide bonds between its heavy

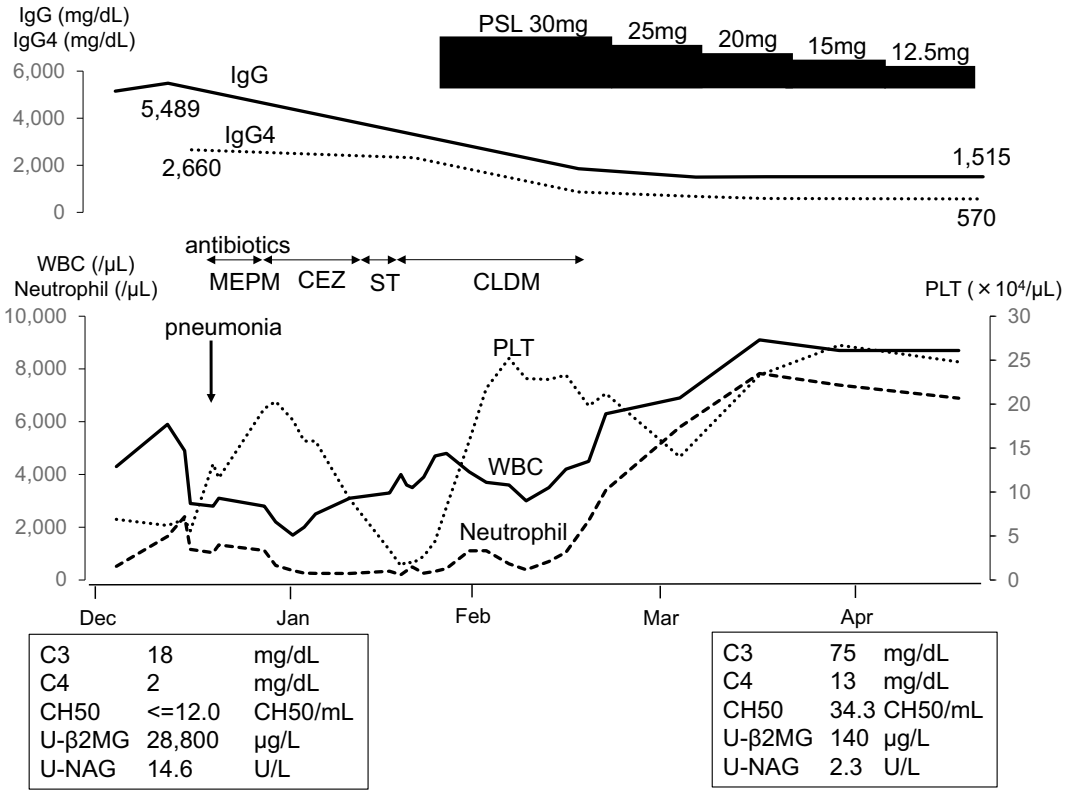

Figure 3. Changes in the titer of anti-neutrophil autoantibodies. Treatment with prednisolone resulted in increases in the patient's neutrophil and platelet counts and reductions in his serum levels of IgG and IgG4. U-β_2MG: urinary β_2-microglobulin, U-NAG: urinary N-acetyl-β-D-glucosaminidase, PLT: platelets, PSL: prednisolone

chains. Once Fab-arm exchange occurs, the molecular features of the IgG4 antibodies change, making them bispecific but mono-valent antibodies. In addition, the Fc portion of the IgG4 molecule has only a limited complement fixing ability. It may therefore be unlikely for IgG4 antibodies to engage in tissue-destructive immune reactions (16). The markedly high IgG4 levels may simply be a reflection of the response to some primary inflammatory stimulus (2).

T cells have recently been suggested to play a role in the pathogenesis of IgG4RD. Some reports have indicated that the production of T helper 2 (Th2) cytokines and regulatory T cell (Treg) cytokines is increased in IgG4RD patients. A recent study showed that a Th17 cell subset is also upregulated in IgG4RD patients (17). These cytokines are suspected to play an important role in the pathogenesis of IgG4 RD. In primary ITP, a T cell imbalance i.e. increases in the numbers of Th1 and Th17 cells and reductions in the numbers of Th2 and Treg cells was reported (18). The T cell subsets involved in AIN have not been studied in detail. Although different T cell subsets are upregulated between primary ITP and IgG4RD, dynamic T cell immune changes might occur in secondary ITP and AIN associated with IgG4RD. These apparent discrepancies in Th1/Th2 and Th17/Treg polarity remain to be elucidated.

IgG4RD is a systemic disease involving multiple organs. Its unique pathological features include the infiltration of IgG4-producing plasma cells on a lymphoproliferative background, which is driven by changes in the frequencies of T-cell subsets and the resultant abnormalities in cytokine production, as described above. Increases in the serum IgG4 level and the number of IgG4-producing cells in the affected tissues is, of course, not the cause of the disease. However, whether this is merely an effect of the skewed T-cell abnormality remains to be elucidated. Further clarification of the pathogenesis of IgG4RD may require examining how and why ostensively immunologically quiet IgG4 is selectively upregulated under such conditions.

The authors state that they have no Conflict of Interest (COI).

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