

IgG4-IgE complex in a patient with IgG4-related disease

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ABSTRACT

Background: IgE concentrations are occasionally elevated in patients with IgG4-related disease (IgG4-RD). In this report, we describe a novel case of IgG4-RD in which IgE concentrations were discordant between measuring reagents.

Case: An 81-year-old man was diagnosed with IgG4-RD and histological autoimmune pancreatitis, which ensued without treatment. The IgE concentrations measured using Elecsys IgE II Immunoassay and Iatroace IgE were 1287.0 IU/mL and 60.9 IU/mL, respectively. IgG4 concentration was 675 mg/dL.

Methods: To identify IgG and IgG4 directly bound to IgE, purification using protein G and anti-IgG4 antibody-conjugated matrixes and size-exclusion high-performance liquid chromatography (HPLC) were performed.

Results: In purification analysis, the IgE concentration of the flow-through and bound fractions were 6.8 IU/mL (10.8%) and 56.2 IU/mL (89.2%) for IgG purification and 6.8 IU/mL (12.2%) and 49.0 IU/mL (87.8%) for IgG4 purification. IgE was eluted as a single peak (640 kDa) using size-exclusion HPLC. In the elution pattern of IgG4, a minor peak (640 kDa) and a major peak (170 kDa) were observed. These results indicate that IgG4 binds to IgE and forms a complex, resulting in a discrepancy between reagents.

Conclusions: In this report, we present an IgG4-IgE complex in a patient with IgG4-RD, which affected the discrepancy in IgE concentrations between IgE reagents. This report points to the significance of increased IgE production in IgG4-RD.

1. Introduction

IgG4 accounts for <5% of total IgG in healthy subjects [1], but the proportion of IgG4 increases, and its concentration can be significantly increased. Hamano et al. [2] reported for the first time that IgG4 concentrations were elevated in the serum of patients with sclerosing pancreatitis. Kamisawa et al. [3] proposed a novel systemic IgG4-related disease (IgG4-RD). The disease was initially recognized in the pancreas, now summered IgG4-RD, and recognized as a chronic fibroinflammatory disorder.

In IgG-RD patients, IgE concentrations have been reported to be predominantly elevated compared to normal subjects, suggesting that IgE-mediated allergic reactions occur in most patients and may be used for the diagnosis and prediction of recurrence [4]. Zhou et al. [5] demonstrated that IgE concentrations at baseline were high in patients with IgG4-RD and were associated with disease relapse. Moreover, Torre

et al. [6] suggested in a large cohort that the pathogenesis of IgG4-RD itself contributes to elevated IgE concentrations even in the absence of atopy. However, the physiological implications of elevated IgE concentrations in IgG4-RD patients are not known.

In this study, we report a patient with IgG4-RD whose IgE concentrations were discrepant between reagents when evaluating different IgE assay reagents.

1.1. Case presentation

An 81-year-old man underwent distal pancreatectomy with en bloc celiac resection for pancreatic cancer 13 years previously. Based on the histopathological findings, the patient was diagnosed with IgG4-RD and histological autoimmune pancreatitis (AIP). The patient was followed up without any treatment. At regular follow-up, the IgE concentrations measured using Elecsys IgE II Immunoassay (reference interval: <232.0

Abbreviations: IgG4-RD, IgG4-related disease; HPLC, high-performance liquid chromatography; AIP, autoimmune pancreatitis.

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IU/mL, Roche Diagnostics, Basel Switzerland) on a cobas e801 and Iatroace IgE (reference interval: <360.9 IU/mL, LSI medicine, Tokyo, Japan) on a BM8040 analyzer (JEOL, Tokyo, Japan) were 1287.0 IU/mL and 60.9 IU/mL, respectively. Both IgE reagents are traceable to 2nd International Reference Preparation WHO Reference Standard 75/502. The IgG4 concentration measured using N-assay LA IgG4 Nittobo (Nittobo, Tokyo, Japan) was 675 mg/dL. The case was compared with control patient whose IgE concentrations were not significantly different from 770.0 IU/mL and 501.0 IU/mL measured using Elecsys and Iatroace. IgG4 for the control patient was 968 mg/dL. Patient characteristics are listed in Table 1.

1.2. Methods

1.2.1. Purification of IgG and IgG4

IgG and IgG4 in the serum were purified using Protein G Sepharose 4 Fast Flow (Cytiva, Tokyo, Japan) and CaptureSelect™ IgG4 (Hu) Affinity Matrix (Thermo Fisher Scientific, MA, USA). Briefly, 100 µL in IgG purification and 40 µL in IgG4 purification of the serum was diluted 1:3 in 20mM sodium phosphate buffer (pH 7.0) and 1:9 in 100 mM sodium phosphate buffer (pH 7.4) and mixed with 100 µL of the matrixes. After incubation, matrixes were washed with sodium phosphate buffer and eluted with 1 M glycine-HCl (pH 3.0). The IgE concentration in flow-through and bound fractions were measured.

1.2.2. Size-exclusion HPLC analysis

Size-exclusion high-performance liquid chromatography (HPLC) was performed on a Superdex 200 Increase 10/300 GL column (10 × 300 mm, Cytiva, Tokyo, Japan). Five hundred microliters of serum were injected. The elution buffer consisted of 150 mM NaCl and 10 mM sodium phosphate buffer (pH 7.4). The flow rate was 1.2 mL/min, and the fraction volume was 0.5 mL. Protein concentrations were monitored at 280 nm using the AKTA Explorer 100 System (GE Healthcare, Buckinghamshire, England). The concentrations of IgE and IgG4 in each fraction were measured.

2. Results and discussion

We first suspected that IgG, especially IgG4, which was extremely high at 675 mg/dL, might have affected IgE measurement. To identify IgG and IgG4 directly bound to IgE, we performed purification using matrixes conjugated with protein G and an anti-IgG4 antibody. In the purification analysis, the IgE concentrations of the flow-through and bound fractions were 6.8 IU/mL (10.8%) and 56.2 IU/mL (89.2%) in IgG purification and 6.8 IU/mL (12.2%) and 49.0 IU/mL (87.8%) in IgG4 purification, respectively (Table 2). These results indicated that most IgE was bound to IgG, and the subclass was IgG4. In the control patient, the percentage of IgE bound to IgG and IgG4 were 21.8% and 0.8%, respectively.

To confirm that the IgE and IgG4 molecules were bound to each other, size-exclusion HPLC was performed. The elution pattern on HPLC analysis showed that IgE was separated as a single peak (640kDa) (Fig. 1A). In addition, IgG4 was separated into minor (640kDa) and

Table 2

Purification of IgG and IgG4.

Purification	Fractions	Case patient		Control patient	
		IgE concentration (IU/mL)	Rate (%)	IgE concentration (IU/mL)	Rate (%)
IgG	Flow-through	6.8	10.8	85.0	78.2
	Bind	56.2	89.2	23.7	21.8
IgG4	Flow-through	6.8	12.2	200.8	99.2
	Bind	49.0	87.8	1.6	0.8

*IgE concentration measured using Iatroace IgE.

major (170kDa) peaks. The elution volume of the minor IgG4 peak corresponded to that of the IgE peak. These results demonstrated that IgG4 binds to IgE and forms a complex. Moreover, IgE concentrations measured using Iatroace IgE were lower than those measured using Elecsys IgE II. These results indicate that IgG4 and IgE form a complex, which inhibits the antibodies in the reagent from binding to IgE, resulting in a low IgE concentration of Iatroace IgE.

In the control patient with IgG4-RD, whose IgG4 was 968 mg/dL, the elution volumes of the IgE peaks differed between the two reagents (Fig. 1B). The peak of IgE was monitored using Elecsys IgE eluted earlier than the peak using Iatroace IgE. This result suggests that IgE is present in various forms in the plasma, affecting the differences in IgE measurements. Furthermore, there was no peak of IgG4, which coincided with the elution peak of IgE. This result supports the purification analysis demonstrating that IgG4 is not bound to IgE (Table 2). Furthermore, this result may indicate that IgE binds to some molecules that are not IgG4 or form a multimeric complex.

Some reports have described the immune complexes for IgE in patients with atopic dermatitis [7] and rheumatoid arthritis [8]. Carini et al. [9] revealed that among the four IgG subclasses, Ig4 reacted strongly with several IgE myeloma proteins. This result supports our finding that most IgE binds with IgG, the majority of which is IgG4. For the control patient, about 20% of IgE was bound to IgG, but the IgG subclass was other than IgG4. On the basis of these results, we believe that most IgE exists as IgG4-IgE complexes when activated IgG4 is bound to IgE.

Kamisawa et al. [10] reported that allergic symptoms were detected in about half of the patients with AIP, suggesting that an allergic mechanism may be involved in the development of AIP in these patients. However, it has been reported that in a large cohort of IgG4-RD patients, mean IgE concentrations were similar in both atopic and non-atopic patients [6], and elevated IgE concentrations were found in non-atopic patients with IgG4-RD kidney disease [11]. These results suggest that the inflammatory environment of IgG4-RD may sustain elevated IgE regardless of antigenic triggers. Although the production of IgG4 and IgE has been related to T helper 2 cells [12,13], the significance and relationship between IgG4 and IgE are still not well understood. Moreover, whether IgG4-IgE is specifically found in IgG4-RD and its significance has not been well evaluated. Therefore, further analysis with a larger number of cases is needed to clarify the clinical significance of IgG4-IgE complexes.

Since IgG4-RD is characterized by elevated IgG4 concentration and increased IgG4⁺ plasma cells in several organs, the specificities and pathologic roles of IgG4 remain unclear. Shiokawa et al. [14] identified the target antigen in autoimmune pancreatitis as laminin 511. Lowiek et al. [15] reported that annexin A11 is an autoantigen of IgG4 in patients with IgG4-RD. Although these previous reports identified the target of IgG4 antibody, to the best of our knowledge, this report is the first to demonstrate the presence of IgG4-IgE complex in a patient with IgG4-RD and the possibility that IgG4 recognizes IgE as an antigen. Therefore, this report may provide a clue to the significance of increased IgE production in IgG4-RD.

Table 1

Patient characteristics.

	Case patient	Control patient
Age	81	60
Sex	male	female
Other autoimmune diseases	AIP	Non
eGFR (mL/min/1.73 m ²)	51.0	76.4
IgG (mg/dL)	2488	2469
IgG4 (mg/dL)	675	968
IgA (mg/dL)	280	76
IgM (mg/dL)	31	69

AIP, autoimmune pancreatitis.

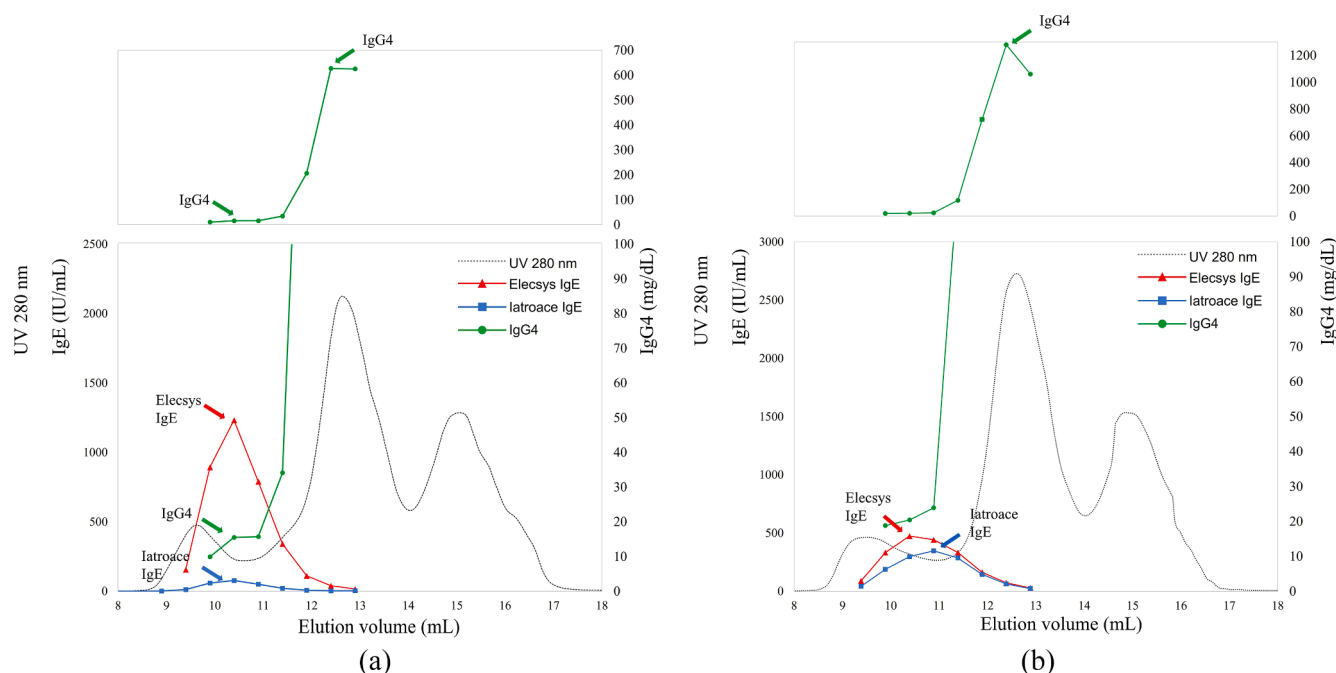


Fig. 1. Elution profiles of IgE, IgG4, and protein obtained by HPLC on a Superdex 200 Increase 10/300 GL column. The red and blue solid lines indicate IgE measured using Elecsys (triangle) and Iatroace (square), respectively. The solid green line indicates IgG4 (circle). The dotted line indicates the protein. (A) The patient whose IgE concentrations were 1287.0 IU/mL and 60.9 IU/mL were measured using Elecsys and Iatroace. The IgG4 concentration was 675 mg/dL. (B) The control patient whose IgE concentrations were 770.0 IU/mL and 501.0 IU/mL measured using Elecsys and Iatroace. The IgG4 concentration was 968 mg/dL.

3. Conclusion

We demonstrated that IgG4 and IgE bound to form a complex, resulting in discrepant IgE concentrations. Further studies are needed to clarify whether this result is unique to this case, or whether it is observed in more patients.

CRediT authorship contribution statement

Keiichi Nakano: Conceptualization, Methodology, Writing – original draft. **Junichi Sugita:** Conceptualization, Writing – original draft. **Naoki Mafune:** Methodology, Formal analysis. **Masanori Seimiya:** Conceptualization, Writing – review & editing, Data curation. **Keiko Yasuda:** Resources, Methodology. **Chiaki Watanabe:** Resources, Project administration. **Takanori Teshima:** Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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