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ORIGINAL ARTICLE

Increased number of IgG4-positive plasma cells in chronic rhinosinusitis

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ABSTRACT

Conclusion: High levels of IgG4-positive plasma cells were observed in tissue samples from ~30% of patients with chronic rhinosinusitis who satisfied the comprehensive diagnostic criteria for IgG4-related disease. Detection of increased numbers of IgG4-positive plasma cells in the nasal cavity or paranasal sinuses might not be sufficient to make a diagnosis of IgG4-related rhinosinusitis, and a comprehensive evaluation is required.

Objectives: This study aimed to clarify the clinicopathological characteristics of IgG4-positive plasma cells in patients with chronic rhinosinusitis.

Method: This study examined nasal mucosal specimens from 35 patients and assigned them to high-IgG4 and low-IgG4 groups based on infiltration of IgG4-positive plasma cells. It compared the pathological characteristics of the two groups, including the presence of fibrosis, phlebitis, hyperplasia of the nasal glands and infiltration of inflammatory cells.

Results: No cases of chronic rhinosinusitis showed storiform fibrosis or obliterative phlebitis. The mean number of IgG4-positive plasma cells in samples from all patients was 29.8 ± 40.3 /high-power field. Eleven of the 35 cases (31.4%) were classified as high-IgG4. Hyperplasia of the nasal glands was observed significantly more frequently in the high-IgG4 group than in the low-IgG4 group ($p = .03$).

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Introduction

IgG4-related disease (IgG4-RD) is a recently recognized systemic immune-mediated disease characterized by dense lymphoplasmacytic infiltration, storiform fibrosis, and elevated serum IgG4 levels [1]. Comprehensive criteria for the diagnosis of IgG4-RD were proposed in 2011 [2]. IgG4-RD may occur, either synchronously or metachronously, in a variety of organs, and may be present in patients with a range of diseases, including chronic sialadenitis (also known as Mikulicz's disease or Küttner's tumour), autoimmune pancreatitis, hypophysitis, Riedel's thyroiditis, interstitial pneumonitis, interstitial nephritis, prostatitis, lymphadenopathy, retroperitoneal fibrosis, inflammatory aortic aneurysm, and inflammatory pseudotumor [3].

Some IgG4-RD patients complain of nasal symptoms such as obstruction, crusting, and hyposmia. Several groups, including ours, have recently reported sinonasal manifestations of IgG4-RD [4–10] and have suggested that IgG4-RD might target the nasal cavity or paranasal sinuses. However, the clinical and pathological features of IgG4-RD-associated nasal lesions remain controversial, and whether IgG4-related rhinosinusitis is a distinct, independent disease is still unclear. A consensus statement on the pathology of IgG4-RD does not currently include rhinosinusitis, and the

presence of IgG4-positive plasma cell infiltrates in biopsy specimens is not specific for IgG4-RD [11]. Extensive infiltration by IgG4-positive plasma cells has been described in common mimickers of IgG4-RD, including other inflammatory conditions and malignancies [12]. However, the relative abundance of IgG4-positive plasma cells in the nasal cavity and paranasal sinuses of patients with non-specific inflammatory conditions has not been clarified. Thus, in the present study, we investigated the clinicopathological characteristics of IgG4-positive plasma cells in patients with chronic rhinosinusitis.

Materials and methods

Patients

We analysed 35 patients with chronic rhinosinusitis who underwent endoscopic sinus surgery for sinusitis, including fungal rhinosinusitis, at Tokyo Metropolitan Geriatric Hospital from January 2013 to December 2014. Nasal mucosal tissues obtained from the middle nasal meatus during the operation were examined. The clinical data were obtained by retrospective analysis of patient records. Although patient serum IgG4 levels were not evaluated, none of the patients were suspected of having IgG4-RD

during the observation period. Computed tomography (CT) findings were scored according to the Lund–Mackay staging system [13].

Pathological examination and immunohistochemistry

Tissue samples were fixed with 15% formalin and embedded in paraffin. A 3- μ m-thick section was cut from each paraffin-embedded tissue block, and the sections were stained with haematoxylin and eosin (H&E) and Elastica van Gieson stain. Immunohistochemical staining was performed by incubation with the following primary antibodies: polyclonal rabbit anti-human IgG antibody (Dako, Glostrup, Denmark), polyclonal sheep anti-human IgG4 antibody (The Binding Site, Birmingham, UK), polyclonal rabbit anti-human KIT antibody (Nichirei, Tokyo, Japan), monoclonal rabbit anti-human CD3 antibody (SP7, Nichirei), monoclonal mouse anti-human CD4 antibody (1F6, Nichirei), monoclonal mouse anti-human CD8 antibody (C8/144B, Nichirei), and monoclonal mouse anti-human CD20 antibody (L26, Nichirei). Bound antibodies were detected by incubation of sections with Histofine simple stain MAX PO (Nichirei) and diaminobenzidine substrate (Nichirei). The sections were then counterstained with haematoxylin. Negative control tissue sections were prepared in the same manner except that the primary antibody was omitted.

Evaluation criteria

IgG4- and total IgG-positive plasma cells were counted in five high-power fields (HPFs), and the mean values were used to calculate the ratio of IgG4- to IgG-positive plasma cells. We counted areas of intense IgG4 focus ('hot spots'). We segregated the patients into two groups (high-IgG4 and low-IgG4) based on their IgG4 plasma cell numbers. In accordance with the comprehensive diagnostic criteria for IgG4-RD [2011], patients were assigned to the high-IgG4 group if (1) the mean number of IgG4-positive plasma cells per HPF of the biopsy sample was >10, and (2) the ratio of IgG4- to IgG-positive plasma cells was >40% [2].

We also evaluated fibrosis, obliterative phlebitis, and hyperplasia of the nasal glands by Elastica van Gieson staining. Lymphocyte and plasma cell infiltration was evaluated in H&E-stained sections and classified as mild (1+), moderate (2+), and severe (3+). For statistical analysis, the moderate and severe groups were combined and compared with

the mild group. Neutrophil and eosinophil infiltration was evaluated and classified as negative (–), mild (1+), and moderate (2+). For statistical analysis, the mild and moderate groups were combined and compared with the negative group.

All parameters were evaluated independently by two investigators (KO and YM), and the mean values were used for statistical analyses.

Statistical analysis

Data are expressed as the mean $\pm$ standard deviation. Fisher's exact test was used for comparison of categorical data, and the Mann–Whitney *U*-test was used to determine the distribution of continuous variables. *P*-values <.05 (two-sided) were considered statistically significant.

Ethics

This study was approved by the Institutional Review Board of Tokyo Metropolitan Geriatric Hospital (No. 260213). Informed written consent was obtained from all patients for the use of the tissue specimens in this study.

Results

Clinical features of patients in the high- and low-IgG4 groups

Patients in the high-IgG4 ($n=11$) and low-IgG4 ($n=24$) groups showed no differences in age, sex, CT score, past history of allergy, blood eosinophil counts, or clinical and pathological diagnoses (Table 1).

Pathological findings and IgG4-positive plasma cell infiltration in samples from patients with chronic rhinosinusitis

The mean number of IgG4-positive plasma cells in tissue samples from all 35 patients was 29.8 ± 40.3 /HPF (range = 0–200/HPF) (Table 2). The mean ratio of IgG4- to IgG-positive plasma cells was $36.6 \pm 29.3\%$ (range = 0–100%). Samples from 22 (62.9%) patients showed marked infiltration of lymphocytes and plasma cells. Seven (20.0%) patients showed mild fibrosis, but storiform fibrosis was absent. No patients showed obliterative phlebitis. Eleven (31.4%) of the

Table 1. Clinical features of the high-IgG4 and low-IgG4 groups.

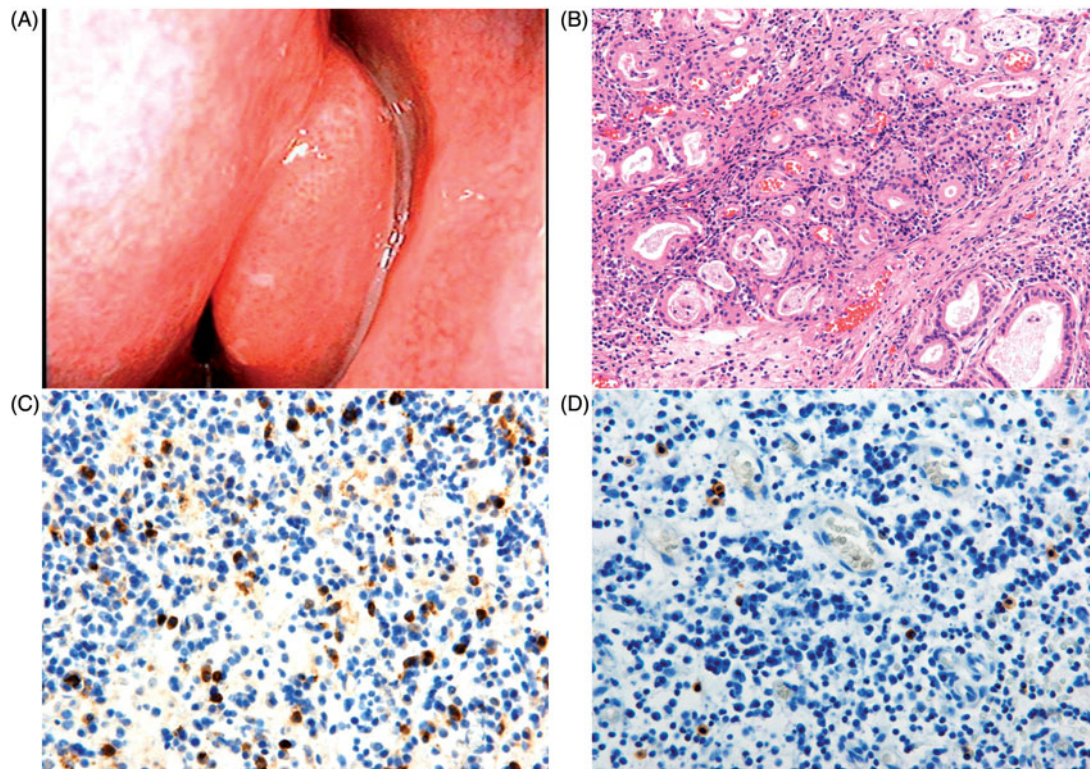
	Total ($n=35$)	High-IgG4 group ($n=11$)	Low-IgG4 group ($n=24$)	<i>p</i> -value
Age, years (mean $\pm$ SD)	75.11 $\pm$ 9.35	75.91 $\pm$ 8.46	74.75 $\pm$ 9.71	.31
Sex, Male/Female	15/20	6/5	9/15	.47
CT score (mean $\pm$ SD)	5.06 $\pm$ 2.92	5.91 $\pm$ 3.99	4.67 $\pm$ 2.15	.35
Diagnosis				
CRS/Fungal sinusitis	23/12	6/5	17/7	1.00
Allergic history				
Allergic rhinitis	7	1	6	.39
Asthma	3	0	3	.54
Eosinophils in the blood (/ μ L) (mean $\pm$ SD)	168.48 $\pm$ 140.46	160.45 $\pm$ 106.59	172.17 $\pm$ 153.56	.36

CRS: chronic rhinosinusitis; CT: computed tomography; SD: standard deviation.

Table 2. Pathological features of the high-IgG4 and low-IgG4 groups.

	Total (n = 35)	High-IgG4 group (n = 11)	Low-IgG4 group (n = 24)	p-value
Number of IgG4-positive cells (/HPF)	29.77 ± 40.27	55.46 ± 51.95	18.00 ± 26.22	0.36
Ratio (%) of IgG4-positive cells to IgG-positive cells	36.64 ± 29.26	64.82 ± 24.30	23.17 ± 20.54	0.04
CD4+ cells dominant/CD8+ cells dominant	3/32	2/9	1/23	0.22
CD3+ cells dominant/CD20+ cells dominant	33/2	11/0	22/2	1.00
Number of KIT-positive cells (/HPF)	6.63 ± 7.83	3.36 ± 2.46	8.12 ± 8.92	0.36
Fibrosis, +/-	7/28	1/10	6/18	0.39
Lymphocyte and plasmacyte infiltration, 2+ and more/1+	22/13	8/3	3/21	0.48
Neutrophil infiltration, +/-	6/29	3/8	3/21	1.00
Eosinophil infiltration, +/-	22/13	8/3	14/10	0.48
Hyperplasia of nasal glands, +/-	15/20	8/3	7/17	0.03

HPF: high-power field.

**Figure 1.** Findings in a representative case from the high-IgG4 group. (A) Mucosal swelling of the ostiomeatal complex with purulent nasal discharge in a 55-year-old man. (B) H&E-stained biopsy specimen of the nasal mucosa showing hyperplasia of the nasal glands and dense inflammatory cell infiltration ($\times 100$). (C) IgG4 immunostaining showed dense IgG4-positive plasma cell infiltration ($\times 400$). (D) KIT immunostaining showed few KIT-positive cells ($\times 400$).

35 patients showed high IgG4-positive plasma cell infiltration, which satisfies the comprehensive diagnostic criteria for IgG4-RD.

Pathological features of tissues from the high- and low-IgG4 groups

The incidence of fibrosis, hyperplasia of the nasal glands, and inflammatory cell infiltration was compared in samples from the high- and low-IgG4 groups (Table 2). Representative findings from a patient in the high-IgG4 group are shown in Figure 1. Hyperplasia of the nasal glands was observed significantly more frequently in the high-IgG4 group than in the low-IgG4 group ($p = .03$, Table 2 and Figure 1(B)). Although fewer KIT-positive cells were detected in the high-IgG4 group than in the low-IgG4 group (Figures 1(D) and 2), the difference was not statistically significant ($p = .36$, Table 2). There

were no significant inter-group differences in any other pathological features (Table 2).

Discussion

Several new observations were made in this study: (1) nasal tissue samples from $\sim 30\%$ of patients with chronic rhinosinusitis showed high infiltration of IgG4-positive plasma cells, which satisfies the comprehensive diagnostic criteria for IgG4-RD (2011) [2]; (2) hyperplasia of the nasal glands was more frequently observed in the high-IgG4 group than in the low-IgG4 group; and (3) none of the patients had storiform fibrosis or obliterative phlebitis.

Our findings support the view that IgG4-related rhinosinusitis cannot be diagnosed solely on the basis of increased IgG4-positive plasma cells in tissue specimens. Furthermore, storiform fibrosis and obliterative phlebitis might increase

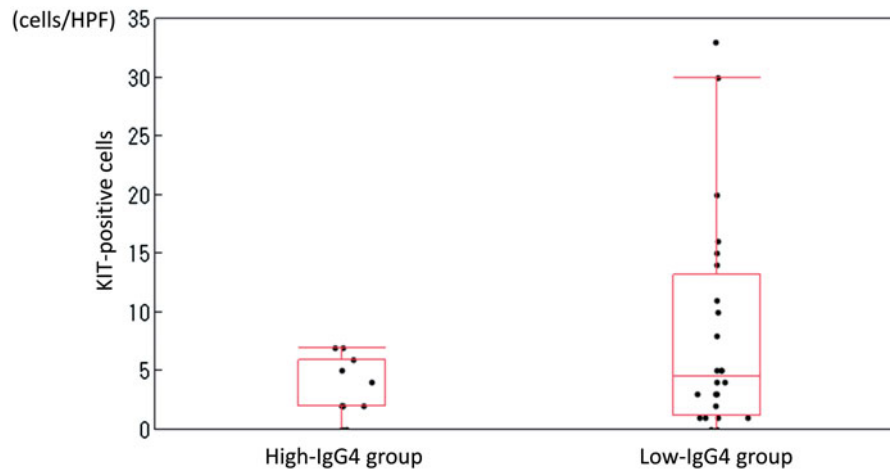


Figure 2. Comparison of the number of KIT-positive cells in the high-IgG4 and low-IgG4 groups. HPF: high-power field.

the diagnostic specificity of IgG4-related sinonasal lesions, as had been reported for autoimmune pancreatitis and sclerosing cholangitis [11]. Although the pathogenesis of IgG4-RD is not yet clear, it has been suggested that type 2 helper T cells (Th2) and regulatory T cells (Tregs) play key roles in IgG4 production [14,15]. The Th2 cytokines IL-4 and IL-13 and the Treg cytokine IL-10 can induce IgG4 class switching [16], and tumor growth factor- β , also a Treg cytokine, can induce tissue fibrosis [14,17]. The roles and pathological significance of IgG4-positive plasma cells in IgG4-RD also remain unclear; however, our study indicates that IgG4-positive plasma cell infiltration in biopsy specimens is a common finding in patients with chronic rhinosinusitis.

We found that hyperplasia of the nasal glands was significantly more common in the high-IgG4 group than in the low-IgG4 group. Hyperplasia of the nasal glands, which induces swelling of the mucosa and active secretion, has also been found in cases of chronic infection or allergy [18]. In contrast, the nasal manifestations of IgG4-RD tend to be crusting and dryness due to the lower number of nasal glands [4,7].

IgE and IgG4 class switching in B-cells are both regulated by the cytokines IL-4, IL-13, and IL-10; therefore, the IgE response is usually accompanied by production of IgG4 [16,19]. This fact suggested that allergic conditions might be associated with abundant IgG4 production. However, in this study, we found that tissues from the high-IgG4 group tended to have fewer KIT-positive cells. The KIT receptor is a marker of mast cells, which are triggered by allergen-specific IgE to release pro-inflammatory mediators. Previous studies have shown that the number of mast cells in the nasal epithelium of allergic patients correlates with allergen exposure, and that, in patients with allergic rhinitis, mast cells migrate to the nasal epithelium during the pollen season [20,21]. In this regard, the pathology underlying the increase of IgG4-positive plasma cells in tissue may differ from the pathology underlying the allergic condition.

Our finding that IgG4-positive plasma cells are increased in patients with non-specific chronic inflammatory conditions of the paranasal sinuses complicates the diagnosis and placement of IgG4-related rhinosinusitis. Suzuki et al. [7]

reported that nasal mucosal tissues obtained from healthy subjects showed a low level of IgG4-positive plasma cell infiltration (1.41/HPF). This suggested that IgG4-positive plasma cells may easily infiltrate regions exposed to inflammation on a daily basis; for example, the oral or nasal cavity. An appropriate level of care should be taken to distinguish IgG4-related sinusitis from mimicking disorders and to avoid over-diagnosis. An accurate diagnosis of IgG4-related rhinosinusitis requires a comprehensive and careful investigation of the characteristic clinical features of nasal manifestation, other characteristic pathological features (such as storiform fibrosis and obliterative phlebitis), accompanying lesions of other organs commonly present in individuals with IgG4-RD, and serological aspects (such as a high serum IgG4 level). In future studies, we plan to conduct a prospective study that includes an investigation of serum IgG4 levels and to further investigate the relevance of other allergic or immune-mediated conditions.

Conclusion

We found that nasal tissues from ~30% of patients with chronic rhinosinusitis had a high level of IgG4-positive plasma cell infiltration, which satisfies the comprehensive diagnostic criteria for IgG4-RD. Our results support the view that IgG4-related rhinosinusitis cannot be diagnosed solely on the basis of increased IgG4-positive plasma cell infiltration, and that a comprehensive approach is required for the diagnosis of IgG4-related rhinosinusitis.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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