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ORIGINAL ARTICLE



Stage classification of IgG4-related dacryoadenitis and sialadenitis by the serum cytokine environment

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ABSTRACT

Objectives: Patients with immunoglobulin-G4 related disease (IgG4-RD) diagnosed according to the comprehensive diagnostic criteria (CDC) show varied therapeutic responses and prognoses. We assumed that there are clinical stages in IgG4-RD and have verified it using serum cytokine levels in the groups classified by lesion distribution.

Methods: Definite IgG4-related dacryoadenitis and sialadenitis (IgG4-DS) cases were divided according to the CDC for IgG4-RD into 11 cases with focal type and 30 cases with systemic type. The levels of serum interleukin (IL)-4, IL-5, IL-6, IL-10, IL-13, IL-15, IL-21, interferon (IFN)- α , IFN- γ , tumor necrosis factor (TNF)- α , transforming growth factor (TGF)- β 1, and monocyte chemoattractant protein (MCP)-1 were measured in healthy controls, allergic patients, probable IgG4-RD cases, and focal and systemic type cases. The cytokine environment was analyzed in each group. The 52 definite IgG4-RD cases were next classified into four groups with cluster analysis in terms of therapeutic responses and prognosis. The relationships between each cytokine level and therapeutic responses were also analyzed.

Results: Both serum IL-5 and IFN- α concentrations were very low in healthy controls, but they increased in the allergic cases, probable cases, and focal and systemic type cases. The level of serum IL-5 was significantly higher in definite cases than in healthy controls. The serum IL-5 level was also significantly increased in the groups with a poor prognosis than in the good prognosis group.

Conclusion: These results suggest that there are clinical stages in IgG4-RD, and serum IL-5 play roles in the pathogenesis of IgG4-RD.

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interferon- α ; interleukin-5;
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Introduction

Immunoglobulin G4-related disease (IgG4-RD) is a systemic disease characterized by abundant infiltration of IgG4-bearing plasma cells with fibrosis in the involved organs and elevated serum IgG4 levels [1]. Information relevant to clinical practice has been gradually accumulated. We established a registry system for IgG4-RD, especially IgG4-related dacryoadenitis and sialadenitis (IgG4-DS), in the SMART registry [2]. It shows that 60% of the patients with IgG4-DS have other organ involvement, and the remaining 40% present with only dacryoadenitis and sialadenitis, so-called focal type. Furthermore, there are some cases that satisfy the histopathological items of the comprehensive diagnostic criteria (CDC) for IgG4-RD, but without elevation of serum IgG4 levels; they are the so-called probable IgG4-RD cases [3]. We recently saw several untreated cases that shifted from probable IgG4-RD to the focal type of definite IgG4-RD or from the focal type to the systemic type within a few years. We assumed that there are clinical stages of IgG4-RD

and verified it using serum cytokine levels in the groups classified by lesion distribution.

Methods

Patients and controls

The subjects were 55 cases with IgG4-RD who visited Sapporo Medical University Hospital between April 2010 and March 2017. All cases underwent submandibular gland biopsy, and they were diagnosed as definite or probable cases according to the CDC for IgG4-RD. Forty-one definite cases were evaluated by the staging classification and divided into focal type (only dacryoadenitis and sialadenitis) and systemic type (with other organ involvement) by ¹⁸F-fluorodeoxyglucose positron emission tomography (FDG-PET) and enhanced computed tomography (CT). Because abnormal uptakes in the hilar lymph nodes were often detected in IgG4-DS, the cases that presented with only these findings were included as focal types. All probable cases presented

without other organ involvement. In the analysis of the relationships between the cytokine levels and therapeutic responses, 52 definite cases of IgG4-DS were classified into four groups. Using the computer, hierarchical cluster analysis was performed with the following clinical continuous variables: age at onset, peripheral eosinophil count, and serum IgG, IgG4, IgE, C-reactive protein (CRP), and complement levels at the first visit. We found that both Cluster 1 patients, who presented with prominent hypergammaglobulinemia, elevated levels of serum IgG4, and hypocomplementemia, and Cluster 3 patients, with younger onset and serum levels of IgG, IgG4, and IgE and peripheral eosinophil counts lower than the other clusters, had a high frequency of relapse. The Cluster 2 patients showed eosinophilia and elevated levels of serum IgG, IgG4, IgE, and CRP. The Cluster 4 patients with elder onset and low peripheral eosinophil counts showed good responses to glucocorticoids, with high rates of drug-free remission [4]. The serum cytokine concentrations were analyzed in each cluster group. The control group consisted of 10 healthy persons and 3 with an allergic condition (hay fever). The serum samples of the patients before treatment and the healthy controls were analyzed.

Measurements of serum cytokine levels

Serum cytokine levels were analyzed with the following enzyme-linked immunosorbent assay (ELISA) kits manufactured by Abcam (Cambridge, UK): IL-4, Human IL-4 SimpleStep ELISA[®] Kit, ab215089; IL-5, Human IL-5 SimpleStep ELISA[®] Kit, ab215536; IL-6, IL-6 (interleukin-6) Human ELISA Kit, ab46027; IL-10, IL-10 (interleukin-10) Human ELISA Kit, ab100549; IL-13, IL-13 (Interleukin-13) Human ELISA Kit, ab100553; IL-15, IL-15 (Interleukin-15) Human ELISA Kit, ab100554; IL-21, IL-21 (Interleukin-21) Human ELISA Kit, ab119542; IFN- α , Human Interferon Alpha-1 SimpleStep ELISA[®] Kit, ab213479; IFN- γ , Interferon- γ (IFNG) Human SimpleStep ELISA[®] Kit, ab174443; TNF- α , TNF- α Human SimpleStep ELISA[®] Kit, ab181421; TGF- β , TGF Beta-1 Human ELISA Kit, ab100647; and MCP-1, MCP1 (CCL2) Human SimpleStep ELISA[®] Kit, ab179886. Comparative analyses were conducted between each group.

Statistical analysis

Pairwise comparisons for categorical variables between groups were made using the chi-squared test or Fisher's exact test, as appropriate. Student's *t*-test was used to compare continuous variables between groups. *p* values < .05 were considered significant. All statistical analyses were performed using Multivariate analysis for Mac version 3 software (ESUMI Co. Ltd., Tokyo, Japan).

Ethical considerations

All study-related recruitment and procedures were approved by the Sapporo Medical University Hospital Institutional

Review Board (292-46). Study participants provided their written, informed consent.

Results

Patient profile

The 55 patients with IgG4-RD included 29 males and 26 females; the three probable cases were all females. The mean age of the definite patients was 58.3 ± 12.8 [standard deviation (SD)] years, and that of probable patients was 57.0 ± 9.2 years. The average serum IgG levels in definite and probable patients were 2604.8 ± 1526.3 (SD) mg/dl and 1270.3 ± 128.1 mg/dl, respectively ($p < .001$). The average serum IgG4 levels in definite and probable patients were 890.0 ± 749.1 mg/dl and 63.3 ± 51.2 mg/dl, respectively ($p < .001$) (Supplementary Table S1).

The group by cluster analysis comprised 13 patients in Cluster 1, 11 in Cluster 2, and 14 each in Clusters 3 and 4. The mean age in each group was: Cluster 1, 58.6 ± 8.7 (SD) years; Cluster 2, 57.2 ± 6.8 years; Cluster 3, 42.0 ± 10.6 years; and Cluster 4, 67.6 ± 7.5 years. The average serum IgG levels were 4664.5 ± 1758.9 (SD) mg/dl in Cluster 1, 2248.1 ± 488.7 mg/dl in Cluster 2, 1674.0 ± 273.7 mg/dl in Cluster 3, and 1903.1 ± 459.5 mg/dl in Cluster 4. The average serum IgG4 levels were 1764.0 ± 922.4 mg/dl in Cluster 1, 883.1 ± 427.6 mg/dl in Cluster 2, 498.9 ± 271.7 mg/dl in Cluster 3, and 475.1 ± 296.3 mg/dl in Cluster 4. The maintenance doses of prednisolone were 8.58 ± 1.88 (SD) mg/day in Cluster 1, 5.70 ± 2.75 mg/day in Cluster 2, 5.14 ± 2.41 mg/day in Cluster 3, and 4.85 ± 3.61 mg/day in Cluster 4. The frequencies of treatment with immunosuppressants were: Cluster 1, 41.67%; Cluster 2, 0.00%; Cluster 3, 28.75%; and Cluster 4, 7.14%. The recurrence rates were 25.00% in Cluster 1, 20.00% in Cluster 2, 42.86% in Cluster 3, and 0.00% in Cluster 4. On the other hand, the drug-free rate was 21.43% in Cluster 4 and 0.00% in the other clusters (Supplementary Table S2) [4].

Serum cytokine levels in each group

The serum cytokine levels are shown in Figure 1. Both serum levels of IL-5 and IFN- α were not detected or very low in healthy controls, but they increased gradually in allergic cases, probable IgG4-RD cases, and the focal and systemic types. There was a significant difference between healthy controls and definite IgG4-RD patients ($p < .01$). IL-6, IL-10, and TGF- β were detected only in the systemic type. As for serum IL-4, IL-13, IL-15, TNF- α , and MCP-1 levels, they were higher in the focal type than in the systemic type.

Associations between cytokine levels and therapeutic responses

Cluster 4, which had a good response to glucocorticoids and a good prognosis, showed low serum IL-4, IL-5, IL-6, IFN- α , and TGF- β levels, while the serum IL-10 level was

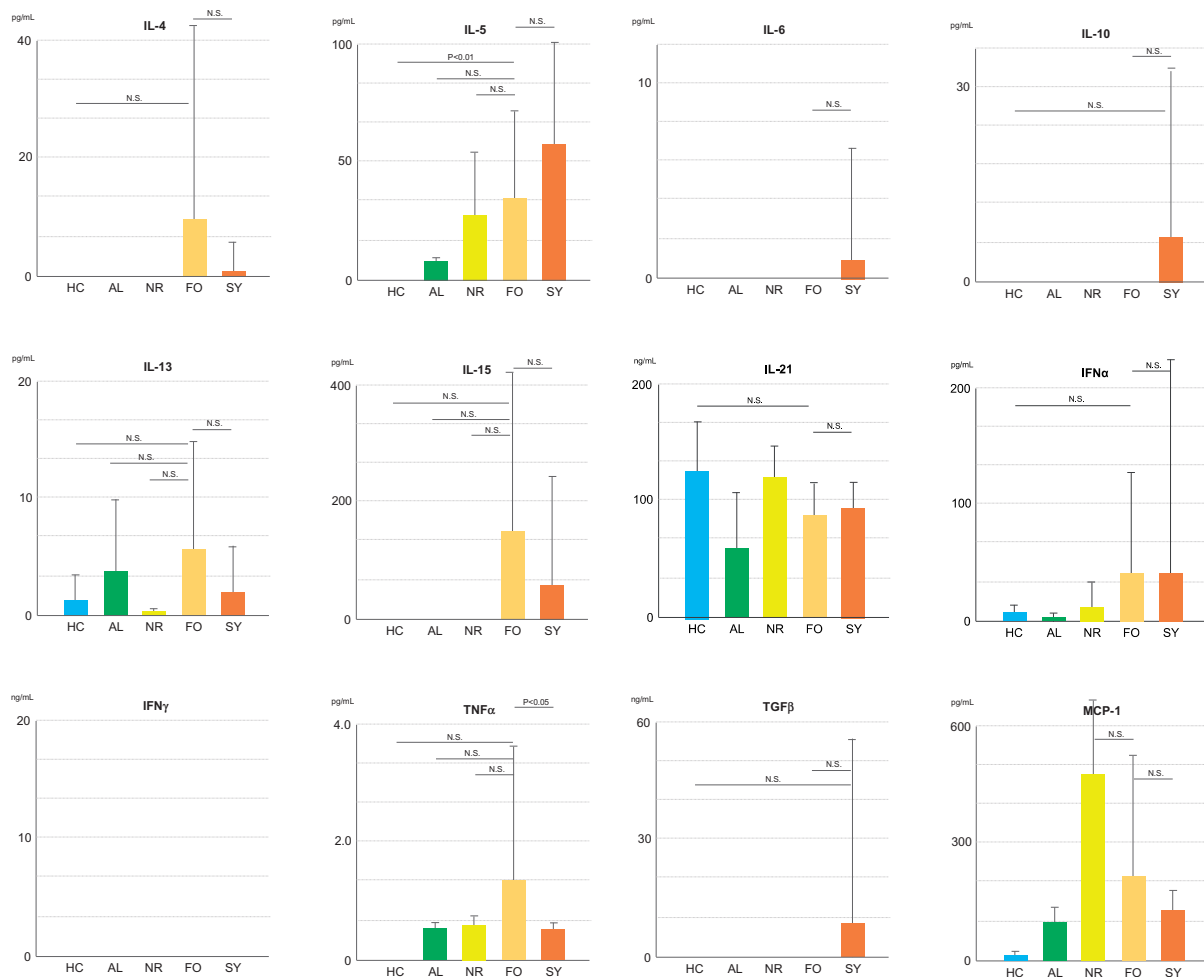


Figure 1. The serum cytokine levels in IgG4-RD cases, allergic cases, and healthy controls. IL-5 and IFN- α are not detected or very low in the sera of healthy controls, but their levels gradually increase in allergic cases, probable IgG4-RD cases, and the focal and systemic types. HC: healthy controls, AL: cases with allergic disorders, mainly allergic rhinitis, NR: normal range of serum IgG4 with IgG4-bearing plasma cells, so called, probable IgG4-RD, FO: focal type of definite IgG4-RD, SY: systemic type of definite IgG4-RD.

increased. On the other hand, serum IL-5, IL-6, IFN- α , and TGF- β levels were increased, and the IL-10 level was low in clusters 1 and 3, which had a poor prognosis. There was a significant difference in IL-5 levels between clusters 1/3 and 4 (Figure 2).

Discussion

Over 10 years have passed since the establishment of the IgG4-RD concept, but more clinical information about IgG4-RD cases is still needed to guide clinical practice. The clinical features, including lesion distribution and treatment response, were found to vary greatly as the number of IgG4-RD cases increased. We noticed that there is a focal type, which is only IgG4-DS, and a systemic type, which is a condition with other organ lesions beyond IgG4-DS. The SMART registry, which consisted mainly of IgG4-DS, showed that 60% of the patients were systemic type [2]. Furthermore, there were probable IgG4-DS cases according to the CDC for IgG4-RD. This phenotype presents with prominent infiltration of IgG4-positive plasmacytes in the involved organs, but no elevation of serum IgG4 levels. We

originally have to divide normal range of serum IgG4 (NR) group into focal and systemic type because Kamisawa [5] reported serum IgG4-negative IgG4-RD with other organ involvements. However in this study, there was no NR belonged to systemic type, so we considered and interpreted NR cases as very early stage of IgG4-DS. Interestingly, we sometimes see the untreated cases shift from focal type to systemic type with increasing serum IgG4 levels. Thus, we assumed and identified several clinical stages in IgG4-RD, and we analyzed the serum cytokine environments in each stage. This is an unprecedented attempt. We reconfirmed the type 2-helper T (Th2)-dominant cytokines in IgG4-DS, as previously reported [6], but we noticed the importance of IL-5 and IFN- α in the pathogenesis of IgG4-DS. In particular, serum IL-5 was not detected in healthy controls, and the serum IL-5 levels were increased as the clinical stage increased. There was a significant difference in serum IL-5 levels between healthy controls and definite IgG4-RD patients. Furthermore, it was interesting that serum IL-5 was detected only in allergic cases. It is known that IL-5 is one of the Th2 cytokines and induces eosinophils. It was previously reported that patients with IgG4-RD often have an atopic diathesis and present with elevated serum IgE levels

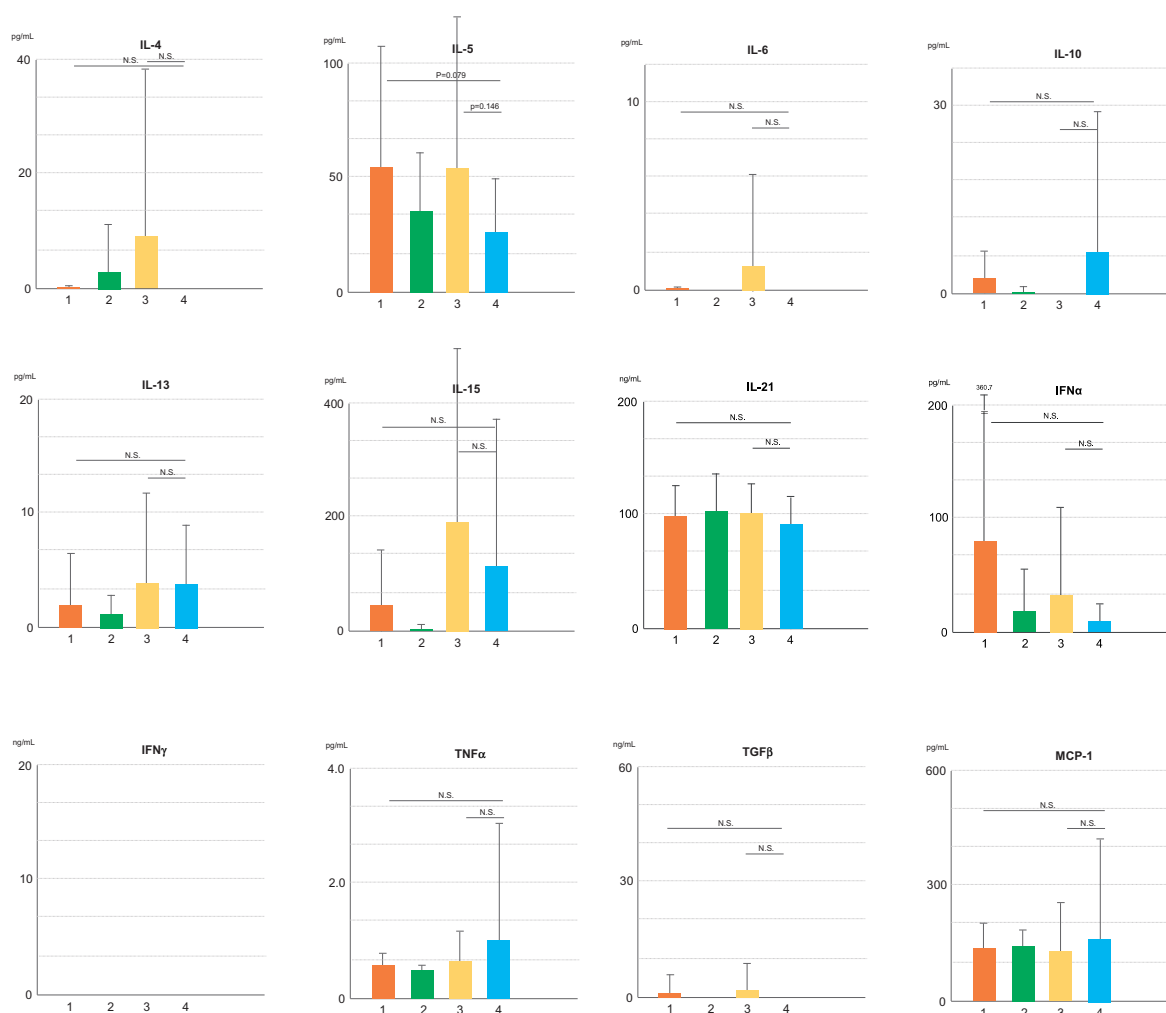


Figure 2. Associations between cytokine levels and therapeutic responses. Cluster 4, which shows a good response to glucocorticoid therapy and a good prognosis, has low serum levels of IL-4, IL-5, IL-6, IFN- α , and TGF- β , and the serum IL-10 level is increased. On the other hand, serum levels of IL-5, IL-6, IFN- α , and TGF- β are increased, and the IL-10 level is low in Clusters 1 and 3, which have a poor prognosis.

and eosinophilia [7,8]. It is considered that IgG4-RD may develop on the background of an atopic predisposition. It was MCP-1 that showed a difference only between patients with an allergic condition and probable IgG4-RD patients. MCP-1 is a chemotactic factor for peripheral monocytes. Abundant infiltration of M2 macrophages is observed in the involved organs [9]. M2 macrophages differentiate from inflammatory monocytes and induce fibrotic lesions. Some triggers induce MCP-1 at the onset of IgG4-RD. It is important to investigate what the triggers are.

Next, with regard to the relationships between serum cytokine levels and therapeutic responses, though the subjects in a previous report were another population, the validation showed the same results. It was also shown that elevated levels of serum IL-5 and IFN- α in the cluster groups were associated with a poor prognosis. The serum IL-5 concentration was significantly higher in the poor prognosis group than in the good prognosis group. The present results suggest that these cytokines are important players in the pathogenesis of IgG4-RD. On the other hand, the serum IL-10 level, which was previously considered to be pathogenic [10], was higher in the good prognosis group. This

suggests that regulatory T cells, which can produce IL-10, may increase reactively and not be involved in the pathogenesis.

There are several limitations in this analysis. First, the number of subjects was very small. This may be the reason why the serum cytokine environment showed only a tendency, with no statistical significance, to be useful for classification, except for IL-5. These analyses will need to be continued with more subjects. Secondly, only serum levels were investigated in the present study; there were only a few cases in which both serum and submandibular glands could be evaluated this time. Our intention is to investigate both samples prospectively in the future.

The present results suggest that there are clinical stages in IgG4-RD, and that the cytokine environment differs by stage (Supplementary Figure S1). The first-line induction therapy for IgG4-RD is uniformly glucocorticoid administration [11]. However, discontinuing steroid therapy is very difficult. The SMART registry showed that only 4.7% of patients attained drug-free remission, and half of the IgG4-RD patients presented with relapse within 7 years from initial treatment. Treatment must be selected according to the

clinical stage. It was found that different cytokines, such as IL-5, IFN- α , and MCP-1, might be therapeutic targets depending on the stage. Personalized medicine and precision medicine should be our targets in dealing with IgG4-RD.

Conclusion

A clinical stage classification was attempted with serum cytokine analysis. The present results suggest that there are clinical stages in IgG4-RD, and that serum IL-5 play roles in the pathogenesis of IgG4-RD. MCP-1 might be involved in the onset of IgG4-RD. Personalized medicine and precision medicine should be our targets in dealing with IgG4-RD.

Conflict of interest

None.

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	Cluster 1	Cluster 2	Cluster 3	Cluster 4
N	13	11	14	14
Male: Female	8: 5	8: 3	2: 12	11: 3
Mean age (yrs.)	58.6±8.7	57.2±6.8	42.0±10.6	67.6±7.5
IgG (mg/dL)	4664.5±1758.9	2248.1±488.7	1674.0±273.7	1903.1±459.5
IgG4 (mg/dL)	1764.0±922.4	883.1±427.6	498.9±271.7	475.1±296.3
Maintein dose (mg/day)	8.58±1.88	5.70±2.75	5.14±2.41	4.85±3.61
Immunosuppressants (%)	41.67	0.00	28.57	7.14
Relapse rate (%)	25.00	20.00	42.86	0.00
Drug-free rate (%)	0.00	0.00	0.00	21.43

	HC	AL	NR	G4
N	10	3	3	52
Male: Female	4: 6	0: 3	0: 3	29: 26
Mean age (yrs.)	59.0±10.2	58.3±10.8	57.0±9.2	58.3±12.8
IgG (mg/dL)	1442.7±158.5	1254.0±56.7	1270.3±128.1	2604.8±1526.3
IgG4 (mg/dL)	23.1±12.5	35.3±14.0	63.3±51.2	890.0±749.1

