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Serum IgG levels demonstrate seasonal change in connective tissue diseases: a large-scale analysis for four years in Japanese

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Abstract

Hypergammaglobulinemia is often found in patients with autoimmune diseases such as systemic lupus erythematosus (SLE), and its level may correlate with disease activity. However, it is unclear whether IgG displays seasonal changes. We analyzed the seasonal change in serum IgG using 450 connective tissue disease patients. The serum IgG levels in summer were compared with those in winter from 2006 to 2009. Independent samples from 355 patients were analyzed to confirm results in the first set. The differences in the IgG levels between the two seasons were analyzed in each disease and compared with disease activity. 488 patients without connective tissue disease were analyzed as reference instead of healthy people as control. We found that connective tissue disease patients tended to show higher levels of serum IgG in summer than in winter every year from 2006 to 2009, while patients without connective tissue disease did not demonstrate such a tendency. We observed this seasonal tendency in each disease. Seasonal changes of serum IgG weakly correlated with those of anti-DNA antibody in SLE patients and those of disease activity score in rheumatoid arthritis patients. The serum IgG levels of patients with connective tissue diseases display seasonal variations. Biological and clinical significance of these variations should be elucidated.

Keywords IgG, connective tissue diseases, rheumatoid arthritis, systemic lupus erythematosus, biomarker, disease activity

Introduction

IgG are a major fraction of immunoglobulins produced by plasma cells in lymphoid organs that comprise ~20% of serum proteins. IgG bind to antigens to induce an inflammatory response or form immune complexes [1]. It is widely known that patients with connective tissue diseases, especially those with Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), or mixed connective tissue disease (MCTD), display hypergammaglobulinemia or a high IgG titer. IgG is used to evaluate the effects of immunosuppressive therapy, and some autoantibody titers are known to be related to disease activity, for example, anti-double strand DNA antibody titers are related to lupus nephritis and its activity [2-4], and a previous report demonstrated that the IgG level is associated with lymphoid infiltration in SS [5]. However, the effects of seasonal changes in the IgG levels of patients with connective tissue diseases have never been considered. During our daily medical practice, we have noticed that connective tissue diseases patients show seasonal changes in their IgG levels regardless of their medication; therefore, we conducted a retrospective chart review of a large number of connective tissue diseases patients at our hospital to verify our hypothesis.

Materials and Methods

Cases and controls

450 Patients with connective tissue diseases who were followed-up at Kyoto University Hospital for whom both summer (July and August) and winter (January and February) IgG measurements were available for the period from 2006 to 2009 were enrolled in this study. They included 195 rheumatoid arthritis (RA), 140 SLE, 46 Systemic Sclerosis (SSc), 19 MCTD, 41 primary SS, and 28 Polymyositis/Dermatomyositis (PM/DM) patients. Independent 355 patients with connective tissue diseases followed-up at Kyoto University were analyzed as the replication of the results in the first set. They included 155 RA, 123 SLE, 33 SSc, 13 MCTD, 32 primary SS, and 23 PM/DM patients. A fraction of patients had more than two connective tissue diseases and overlapping in more than two disease subgroups was allowed. We extracted 488 patients without connective tissue diseases whose IgG data were available from 39,089 outpatients at Kyoto University Hospital on January and February in 2010 and analyzed them as reference instead of healthy people as control. Basic information of each group was shown in Table 1. Connective tissue diseases patients fulfilled the criteria of each disease, namely, ACR criteria for RA[6], SLE[7], SSc[8], Japanese criteria for primary SS[9], criteria for MCTD[10] and criteria for PM

and DM[11]. This study was designed in accordance with Helsinki Declaration and granted by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee. Information of this study is disclosed to all the patients instead of obtaining written informed consent.

IgG levels and disease markers

A retrospective chart review of the enrolled patients was performed to evaluate their serum IgG levels from 2006 to 2009. We obtained disease activity score (DAS) 28 in patients with RA and titers of serum C3, C4, CH50, anti-DNA antibody, and urine protein in patients with SLE from 2006 to 2009. We used data of these markers which were evaluated on the same date as evaluation of IgG.

Statistical analyses

The serum IgG levels in summer were compared with those in winter for each patient from 2006 to 2009. The difference between the two seasons was defined as ΔIgG (ΔIgG =IgGsummer - IgGwinter). The ratio value of positive ΔIgG in each group for each year was compared with the null hypothesis that the ratio is not different from 50% in binomial test. Logistic regression analyses were used to adjust other factors such as age, sex, and treatment. Statistical analyses were performed using R software (http://www.r-project.org/) or SPSS (version 18).

Results

450 patients with connective tissue diseases were selected and had their IgG levels evaluated in a retrospective manner from 2006 to 2009. The connective tissue disease patients showed higher IgG levels in summer than in winter in 2009 (p=0.00070, Table 2). This tendency was kept in other 3 years (Table 2). To confirm these results, we evaluated another independent set containing 355 connective tissue disease patients who were followed-up around the same time as the first set. The second set also showed that the connective tissue disease patients had higher IgG levels in summer than in winter in all the four years (Table 2). When we combined the two datasets, the ratio of positive ΔIgG in the connective tissue disease patients reached significant level in 2006, 2007, and 2009 (Table 2). Although the ratio did not show significant p-value in 2008, the tendency was kept in 2008 (Table 2). Especially SLE within connective tissue diseases demonstrated high ratio value of positive ΔIgG (Table 2).

As we could not obtain the successive serum IgG data from healthy people, we used 488 patients without connective tissue diseases as reference to investigate whether this

IgG seasonal change found in connective tissue disease was observed in general. As a result, we could not find any regular tendency of ratio value of positive ΔIgG in this group (Table 2). Logistic regression analysis using these patients and connective tissue disease patients demonstrated that the positive ΔIgG was associated with to have connective tissue diseases even after adjustment by serum IgG levels at baseline, age, sex, and treatment (Table 3).

Next we analyzed the data with stringent cut off (ΔIgG >10mg/dl as positive change and -10mg/dl >ΔIgG as negative change) to exclude the possibility that just subtle seasonal movement of serum IgG level greatly influenced on the overall ratio value of positive ΔIgG. There is a possibility that change of temperature, humidity and so on between the seasons may influence on machinery to cause subtle change. We observed the same tendency of IgG movement even with stringent cutoff in connective tissue diseases and each connective tissue disease subgroup (Supplementary Table 1). The reference group did not show a regular tendency. When we set more stringent cut off (ΔIgG >100mg/dl as positive change and -100mg/dl >ΔIgG as negative change), we observed the same results in all groups (Supplementary Table 2). From these, we concluded that the seasonal variations of serum IgG we found in the current study were not due to some kinds of errors in machinery or sampling bias.

As the SLE subgroup shows clear seasonal variations of serum IgG among connective tissue diseases and it is a large part of patients with connective tissue diseases in this study, we chose the SLE patients to assess the correlation of ΔIgG with disease activity. The successive data of anti-DNA antibody, C3, C4, CH50, and urine protein were obtained for the SLE patients. We compared the changes of these markers for SLE activity with ΔIgG in the patients. While we could not observe meaningful association between ΔIgG and seasonal change of C3, C4, CH50 or urine protein, we found that ΔIgG was moderately correlated with the seasonal change of anti-DNA antibody in 2009 (rho(Spearman's rank-sum coefficient)=0.35, p=1.6x10^{-5}). We found that this correlation was kept in other three years (rho is 0.30 or larger). Even when we chose SLE patients demonstrating a stringent changes of anti-DNA antibody (Δanti-DNA >=5 IU/ml or -5 IU/ml>=Δanti-DNA), we observed rather strong correlation between the two markers in 2009 (rho=0.47, p=0.013, Figure 1a). We found that this tendency was also seen in other three years (rho is 0.52 or larger).

Next we obtained DAS28 score of 40 RA patients in 2009, the standard measurement to evaluate clinical activity of RA, and analyzed whether ΔIgG correlated with the change of DAS28 or not. We detected moderate correlation between them (rho=0.39, p=0.012, Figure 1b). This correlation was observed in other three years (rho

is 0.28 or larger).

Discussion

It is widely known that many autoimmune diseases are associated with hypergammaglobulinemia mainly consisting of IgG. In some diseases, hypergammaglobulinemia has been suggested to be associated with disease activity, such as lymphoid cell infiltration, treatment responsiveness, and pulmonary arterial hypertension in SS [5, 12]. Another previous report stated that hypergammaglobulinemia in children presenting with SLE-like symptoms is a predictive factor for developing MCTD [13]. However, no previous reports have evaluated the seasonal change in IgG on a large scale. Here, we showed that more than half of patients with connective tissue diseases demonstrated higher IgG levels in summer than in winter and that some of the seasonal variations may correlate with disease activity to some extent.

When we focus on patients with connective tissue diseases whose IgG data were frequently measured to determine two seasons to compare in the preliminary study, the movement of their IgG titers throughout the year suggested that IgG levels in spring and autumn are between the levels in summer and winter (data not shown). This indicates that our comparison between the two seasons in the current study is enough to assess seasonal variation of serum IgG. Moreover, to compare specific two seasons seems good to avoid multiple testing which increases type 1 error of the statistics.

In this study, we analyzed two independent set of samples with connective tissue diseases and found that this tendency of seasonal variation of IgG levels was kept for all the four years. We observed this tendency in each connective tissue disease and in patients with SLE in particular. As we could not obtain IgG data for healthy people, we used data from 488 patients without connective tissue diseases as reference. They have variable diseases from variable departments, such as eczema, IgA nephropathy, HTLV-1 infection, and malignant lymphoma. As a result, they did not show a regular tendency. Although it's much better to use data from age and sex-matched healthy people as control, the result obtained from reference group suggests that the seasonal change of serum IgG levels is not seen in general. Moreover, the logistic regression analysis did not significantly alter the association between seasonal variation of IgG and connective tissue diseases, even with adjustment by age, sex, treatment, and serum IgG level at baseline. When these variations were mainly comprised by very small variations of IgG such as less than 10 mg/dl, some might argue that they are not fully convincing. These small changes may include some machinery errors. However, when we set

stringent cut offs for seasonal variation of serum IgG level, we still observed seasonal changes of serum IgG in connective tissue diseases and its subgroups. This denied the possibility of our results being affected by some tiny changes of IgG levels due to machinery errors between the two seasons.

To analyze the biological meaning of its seasonal change, we compared ΔIgG with changes of levels of anti-DNA antibody, complement, and urine protein in patients with SLE and those of DAS28 in patients with RA. We found that ΔIgG weakly correlated with changes of serum anti-DNA antibody in SLE patients and with those of DAS28 in RA patients. Because anti-DNA antibody is a fraction of IgG, it is reasonable for them to correlate with each other to some extent. However, it is interesting that ΔIgG strongly correlate with stringent change of anti-DNA antibody. These results suggest that ΔIgG reflect changes of disease activity in some fraction of patients with connective tissue diseases, although further analysis is necessary.

Seasonal effects on the onset, relapse, and disease activity of some connective tissue diseases have been reported. In general, SLE activity is believed to increase in spring and summer due to sunlight exposure [14]. However, there are also conflicting reports showing no seasonal change in lupus activity [15-16]. The influence of season on PM/DM is also disputed [17-18]. Therefore, it does not seem that the seasonal IgG changes seen in connective tissue diseases patients are solely due to disease activity. It is possible that the use of additional medications including immunosuppressants or corticosteroids to suppress disease flare-up in winter affected our results. However, logistic regression analysis did not alter the association after adjustment of treatment. Moreover, patients with primary SS, in whom immunosuppressants or corticosteroids are scarcely used, also showed the same tendency, suggesting that this possibility cannot fully explain the phenomena.

The IgG change in the connective tissue diseases patients seemed to be proportional to the temperature throughout the year, but it is unlikely that the change in IgG is caused by temperature changes because the reference group did not show a similar tendency. When we compared the IgG level with the mean temperature in Kyoto, the correlation between them was highly variable from patient to patient (data not shown).

The difference between the patients with connective tissue diseases and those without them might reflect a difference in B cell function between the two groups. To elucidate whether other immunoglobulin fractions act in the same manner as IgG, we analyzed IgA and IgM in a similar manner. However, we could not find any regular tendency of seasonal change in IgA or IgM in either the controls or connective tissue

disease patients (data not shown).

We do not know the underlying mechanisms of this IgG change in connective tissue disease patients. Further analysis should be performed to address this question. To investigate whether this change is related to temperature, it would be interesting and feasible to investigate the ΔIgG in patients with connective tissue diseases in the Southern Hemisphere.

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Conflicts of interest Statement: none

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Figure legend

Figure 1

Correlation between ΔIgG and variation of disease activity in SLE and RA.

Seasonal variation of serum IgG levels was compared with that of anti-DNA antibody in 27 SLE patients (a) or that of DAS28 in 40 RA patients (b). The results in 2009 were shown as a representative.

Table 1. Basic information of patients

	Connective tissue disease	**Non connective tissue disease**
	1st set+2nd set	Reference Set
Number	805	488
Sex	Female:714, Male:91	Female:259, Male:229
Age*	54.3±15.3	45.0±24.8
	1st set	
Number	450	
Sex	Female:402, Male:48	
Age*	54.7±15.4	
	2nd set	
Number	355	
Sex	Female:312, Male:43	
Age*	53.8±15.2	

*Age indicates mean ± standard deviation.

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Table 2. Ratio of patients in each year whose serum IgG levels are higher in summer than in winter.

	2006		2007		2008		2009	
	Positive Ratio	*p*	Positive Ratio	*P*	Positive Ratio	*p*	Positive Ratio	*p*
1st set connective tissue disease	136/236 57.6(51.3-63.9)	0.090	176/298 59.1(53.5-64.6)	0.017	186/349 53.3(48.1-58.5)	0.41	212/346 61.3(56.1-66.4)	0.00070
2nd set connective tissue disease	97/160 60.6(53.1-68.2)	0.046	122/221 55.2(48.6-61.8)	0.29	151/277 54.5(48.6-60.4)	0.31	166/272 61(55.2-66.8)	0.0042
1st set + 2nd set connective tissue disease	233/396 58.8(54.0-63.7)	0.0059	298/519 57.4(53.2-61.7)	0.0087	337/626 53.8(49.9-57.7)	0.18	378/618 61.2(57.3-65.0)	2.7×10^{-6}
RA	91/153 59.5(51.7-67.3)		110/224 49.1(42.6-55.7)		141/275 51.3(45.4-57.2)		159/276 57.6(51.8-63.4)	
SLE	87/145 60.0(52.0-68.0)		116/193 60.1(53.2-67.0)		117/213 54.9(48.2-61.6)		139/213 65.3(58.9-71.7)	
SSc	25/44 56.8(42.2-71.5)		20/45 44.4(29.9-59.0)		31/54 57.4(44.2-70.6)		30/48 62.5(48.8-76.2)	
Primary SS	23/39 59.0(43.5-74.4)		25/35 71.4(56.5-86.4)		25/49 51.0(37.0-65.0)		28/44 63.6(49.4-77.9)	
MCTD	13/18 72.2(51.5-92.9)		17/22 77.3(59.8-94.8)		11/24 45.8(25.9-65.8)		17/27 63(44.7-81.2)	
PM/DM	8/20		22/33		26/41		27/44	

	40.0(18.5-61.5)	66.7(50.6-82.8)	63.4(48.7-78.2)	61.4(47.0-75.8)
Reference Set	76/148	104/199	131/273	149/314
	51.4(43.3-59.4)	52.3(45.3-59.2)	48(42.1-53.9)	47.5(41.9-53.0)

Seasonal change of serum IgG in patients with connective tissue diseases and patients without connective tissue diseases as reference was shown. Patients whose data of IgG change were available were analyzed in each year. While the results in patients with connective tissue diseases in the first set, second set, and combined sets were indicated, those in patients with each connective tissue disease in the combined sets were shown. Positive ratio indicates a ratio value of patients whose IgG levels were higher in summer than in winter in each year. When a patient had more than two connective tissue diseases, overlapping in more than two groups was allowed.

P-values were calculated using the binomial test.

Table 3. Association of connective tissue diseases with positive ΔIgG.

	p	Odds Ratio (95%CI)
IgG in winter*	0.021	0.97(0.95-1.00)
ICS**	0.66	0.93(0.66-1.30)
Corticosteroid	0.50	0.91(0.67-1.22)
Connective Tissue Disease	0.0078	1.58(1.12-2.23)
Age	0.94	1.00(0.99-1.01)
Female	0.043	1.42(1.00-2.00)

Logistic regression analysis was performed using positive ΔIgG as a dependent variable and IgG in winter, usage of immunosuppressant or corticosteroid, having connective tissue diseases, age, and sex as independent variables. The results in 2009 using the data of 618 patients with connective tissue diseases and 314 patients without connective tissue diseases were shown as a representative.

*Odds ratio of IgG in winter indicates Odds Ratio of increase of 100 mg/dl serum IgG.

**immunosuppressant

Figure

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