

Specific IgG for cat allergens in patients with allergic conjunctivitis

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Received: 27 March 2014 / Accepted: 7 August 2014 / Published online: 5 September 2014
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Abstract Immunoglobulin G (IgG) antibodies are involved in type II and type III hypersensitivity. We evaluated the relation between perennial allergic conjunctivitis and serum levels of specific IgG for cat allergens. A prospective study was conducted in patients with seasonal allergic conjunctivitis (seasonal group, $n = 10$), patients with perennial allergic conjunctivitis (perennial group, $n = 10$), and healthy control subjects (control group, $n = 10$). Serum levels of specific IgE and IgG for cat allergens and total tear IgE were measured, and a skin prick test was also performed. In addition, a severity score associated with allergic conjunctivitis was calculated (0–30). The positive rates and scores of for total tear IgE, serum cat-specific IgE, and serum cat-specific IgG were all higher in the seasonal and perennial groups than in the control group (all $p < 0.05$). Serum cat-specific IgG levels were higher in the perennial group than in the seasonal group ($p = 0.0156$), but there was no significant difference

in the grade of cat-specific IgE between the two groups ($p = 0.3008$). On multivariate analysis, the mean wheal diameter for cat allergen was associated with the serum level of cat-specific IgG (not IgE) in all patients [odds ratio (OR) = 31.979, $p < 0.0001$]. Multivariate analysis revealed that the total objective score was strongly associated with serum cat-specific IgG (OR = 23.015, $p < 0.0001$). These findings suggest that specific IgG antibodies may be involved in perennial allergic symptoms caused by indoor allergens such as cat allergens.

Keywords Allergic conjunctivitis · Specific IgE · Specific IgG · Cat · Seasonal allergy · Perennial allergy

Introduction

Immunoglobulins (Igs) are produced to protect the host against various insults. The five primary classes of Igs are termed immunoglobulin A (IgA), IgG, IgM, IgD, and IgE. These five different types of Ig have different antigen-binding properties that provide protection against (or involvement in) specific diseases. For instance, IgE mediates immediate-type I hypersensitivity reactions and is involved in various allergic diseases, such as allergic asthma, allergic rhinitis, and atopic dermatitis [1]. Accordingly, measurement of IgE is widely performed for the clinical diagnosis of allergy, and measurement of allergen-specific IgE

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levels is a useful tool for identifying the causes of allergic reactions [2, 3]. In contrast, IgG mediates type II and type III hypersensitivity reactions, which usually manifest as chronic inflammation. Thus, measurement of IgG can be used as a diagnostic tool for viral infections and inflammatory diseases. IgG has also been reported to be associated with atopic disorders [4] and food allergy [5–7]. Recently, measurement of IgG has been employed as a screening test [8, 9]. However, measurement of the serum IgG level has not yet gained popularity in the assessment of allergic diseases other than food allergies.

Allergic conjunctivitis is one of the most common allergic diseases and includes seasonal, perennial, and atopic conjunctivitis. Immune responses mediated by IgE are important in the pathogenesis of acute seasonal allergy [2, 3], while IgG may be related to indoor allergen-induced perennial allergy. We hypothesized that perennial allergic conjunctivitis might be mediated by specific IgG antibodies associated with indoor allergens. Although allergens derived from cats are a major cause of indoor allergen-induced allergic disease [10], the relation between cat allergens and allergic conjunctivitis has not been described previously. Accordingly, the aims of the present study were (1) to compare sensitization to cat allergens among healthy subjects and patients with seasonal or perennial allergic conjunctivitis, (2) to compare the levels of specific IgG and IgE antibodies for cat allergens with cutaneous reactivity to cat allergens in patients who had perennial allergic conjunctivitis, and (3) to evaluate the relation between serum levels of specific IgG for cat allergens and the features of perennial allergic conjunctivitis.

Materials and methods

Study design

This was a prospective, nonrandomized, cross-sectional, consecutive case series study conducted at Tokyo Women's Medical University Medical Center East Hospital and its affiliated hospitals. This study was performed in accordance with the Helsinki Declaration. Our Institutional Review Board approved the study, and informed consent was obtained from each subject.

Subjects

All subjects had a Schirmer 1 test score >15 mm and normal meibomian glands. None of them had used topical or systemic drugs during the 6-week period before enrollment. We excluded patients with dry eye syndrome, atopic blepharoconjunctivitis, atopic keratoconjunctivitis, and vernal keratoconjunctivitis (VKC), as well as patients wearing contact lenses and those with a history of cataract surgery, corneal refractive surgery, or infectious conjunctivitis. The following three groups of subjects were enrolled in this study (Table 1).

The seasonal group consisted of 10 outpatients with acute seasonal allergic conjunctivitis (4 males and 6 females with a mean age of 27.3 ± 15.4 years; range 5–54 years), while the perennial group consisted of 10 patients with perennial allergic conjunctivitis (4 males and 6 females with a mean age of 28.1 ± 14.7 years; range 5–59 years). We also enrolled an age- and sex-matched control group, comprising 10 healthy non-smoking subjects with no history of allergic disease (3 males and 7 females with a mean age of 28.8 ± 10.7 years; range 12–47 years). The control group was selected from among persons attending our outpatient clinic for eye screening (Table 1).

Diagnosis of allergic conjunctivitis

Allergic conjunctivitis was diagnosed from the findings on slit lamp examination, including conjunctival hyperemia, follicles, and papillae, as well as from symptoms such as ocular itching and tearing. The diagnosis was made by a single ophthalmologist (T.M.) according to published guidelines for the diagnosis and treatment of conjunctivitis [11, 12].

The ocular findings on slit lamp examination were scored at each subject's first visit. Changes of the palpebral conjunctiva (hyperemia, edema, follicles, papillae, and giant papillae), changes of the bulbar conjunctiva (hyperemia and chemosis), changes of the limbus (Trantas' dots and edema), and corneal involvement were classified into four grades (0 = normal, 1+ = mild, 2+ = moderate, and 3+ = severe) [13–15]. Then, the total objective score for allergic conjunctivitis (maximum: 30 points) was calculated as the sum of the scores for the above-mentioned 10 findings [14]. Both eyes were evaluated, but only data for the right eye were used in the present analysis.

Table 1 Clinical profile of the subjects

	Control group	Seasonal group	Perennial group	<i>p</i> value
Number of subjects	10	10	10	–
Gender				
Male/female	3/7	4/6	4/6	NS*
Age (years)	28.8 ± 10.7	27.3 ± 15.4	28.1 ± 14.7	NS**
Cat at home	3	2	2	
Allergy test				
Total tear IgE	0 (0 %)	10 (100 %)	10 (100 %)	<0.0001*
Serum cat-specific IgE	0 (0 %)	1 (10 %)	4 (40 %)	0.0442*
Serum cat-specific IgG	0 (0 %)	1 (10 %)	7 (70 %)	0.0007*
Skin prick test	0 (0 %)	3 (30 %)	7 (70 %)	0.0039*

Number of patients (percentage) or mean standard deviation (SD)

NS not significant

* χ^2 test of independence or Fisher's exact probability test

** One-way analysis of variance and Scheffe's multiple comparison test

Determination of total IgE in tear fluid

Determination of the total IgE level in tear fluid (total tear IgE) was performed with the Allerwatch® test according to the manufacturer's instructions [16–25]. In brief, the filter strip was placed at the lower fornix of the right eye, and was removed when tear fluid reached the control line. Then, the strip was immersed in 100 µl of reaction buffer in a 2.0-ml Eppendorff tube for 10 min at room temperature. The primary antibody (gold-labeled anti-human IgE) bound to IgE in the tear fluid and then was captured by mouse anti-human IgE antibody immobilized at the test line on the strip. Gold-labeled anti-human IgE was also immobilized at a control line on each strip. The intensity of red coloration of the test line depended on the total IgE content of the sample. At normal IgE levels (<2.0 KU/l), no color was detected. The results were classified according to the following three grades: grade 0 = no detectable IgE (no line), grade 1 = low total IgE level (test line weaker than the control line), and grade 2 = high total IgE level (test line similar to or stronger than the control line). Tests were assessed by naked eye observation and then the results were confirmed using Scion Image software (Scion Corporation, Frederick, MD).

Measurement of serum specific IgE for cat allergen

Measurement of specific IgE for cat allergen was performed with the ImmunoCAP Rapid®

immunochromatography assay kit, which is designed to directly measure IgE antibodies targeting cat epithelium/dander (e1). The kit was used according to the manufacturer's instructions [26]. In brief, blood was obtained from a fingertip using a microtainer safety lancet (BD, Tokyo, Japan), and 100 µl of blood was transferred to an ImmunoCAP Rapid® cassette. Specific IgE antibodies in the blood sample reacted with cat antigen at the capture sites during migration through the immunochromatography membrane, creating specific IgE–allergen complexes. At 5 min after sample application, 500 µl of enzyme substrate was added to the substrate reservoir pad. The enzyme, together with a gold-labeled anti-IgE antibody, bound to the antigen–specific IgE antibody complexes at the capture site, and a colored line at the test area indicated the presence of specific IgE antibodies for cat allergens. At 15 min after sample application, the test line was assessed. A red line indicated a positive result, while the absence of color indicated a negative result. The intensity of red coloration of the test line was dependent on the level of specific IgE. Positive or negative results were graded according to the manufacturer's instructions on a scale of 0–2.

Measurement of serum specific IgG for cat allergen

Measurement of specific IgG for cat allergen was performed with an enzyme-linked immunosorbent

Table 2 Results of each immunoglobulin test

	Total tear IgE	Serum cat-specific IgE	Serum cat-specific IgG
Sensitivity (%)	100.0	50.0	80.0
Specificity (%)	50.0	100.0	100.0
False-positive rate (%)	50.0	0.0	0.0
False-negative rate (%)	0.0	50.0	20.0
Positive predictive value (%)	50.0	100.0	100.0
Negative predictive value (%)	100.0	80.0	90.9
Positive likelihood ratio	2.0	-(∞)	-(∞)

Detection of IgE or IgG antibody and positive/negative results of the skin prick test for cat allergen in all subjects ($n = 30$)

assay (ELISA) from US BioTek Laboratories (Seattle, WA). Blood was obtained from a fingertip using a microtainer safety lancet. About 150 μ l of blood was applied to each of the three test strip pads, which were then dried and packed into plastic bags for shipping to US BioTek Laboratories where specific IgG for cat allergen was measured by ELISA [27]. Cat antigen was bound to the surface of an ELISA plate, and whole blood was added to the plate. After antibodies in the blood target to the immobilized antigen on the plate, excess blood was washed off, and anti-human IgG antibody conjugated to horseradish peroxidase (IgG-HRP) was added to detect the bound IgG. After an additional washing step, tetramethylbenzidine was added to detect to develop a blue color by reacting with HRP. The reaction was stopped by adding the stop solution, which turned the color to yellow. Then, the optical density at 450 nm was assessed to determine the amount of antigen-specific IgG [28], and the result was converted into a class score from 0 to 6.

Skin prick test

We performed a skin prick test (SPT) for cat allergen with a single-use sterile lancet (Yayoi Corporation, Tokyo, Japan). A drop of cat dander extract (1:20 wt/vol; Torii Pharmaceutical Co., Ltd., Tokyo, Japan) was applied to the forearm and introduced into the epidermis via lancet puncture. Physiological saline (isotonic sodium chloride solution; Otsuka Pharmaceutical Co., Tokyo, Japan) was employed as the negative control. SPTs were evaluated after 15 min, and a test was considered positive if the mean wheal diameter was ≥ 3 mm.

Statistical analysis

Mean values were compared between two groups with the two-tailed unpaired Student's t test and were

compared among three groups by one-way analysis of variance (ANOVA) and Scheffe's multiple comparison test. Frequencies were analyzed using the χ^2 test of independence or Fisher's exact probability test. Differences of semiquantitative measurements were examined by the two-tailed Mann–Whitney U test for two groups and with the Kruskal–Wallis one-way ANOVA by ranks for three groups. Relations among variables were investigated by calculating Pearson's correlation coefficients, and differences between the coefficients were tested with Fisher's z transformation. Factors associated with the SPT or the total objective score were investigated by multivariate logistic regression analysis, with explanatory variables including the scores for the features of allergic conjunctivitis. The sensitivity, specificity, and positive and negative predictive values were calculated with standard formulae. The level of significance was set at $p < 0.05$. Statistical analysis was performed with SAS System software version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA).

Results

The positive rate of total tear IgE was higher in the seasonal group (100 %) and the perennial group (100 %) than in the control group (0 %; $\chi^2 = 30.0$, $df = 2$, $p < 0.0001$, Fisher's exact probability test; Table 1). Positivity for serum specific IgE and IgG to cat allergens was significantly more frequent in the seasonal group (IgE, 10 %; IgG, 10 %) and the perennial group (IgE, 40 %; IgG, 70 %) than in the control group (IgE, 0 %, $\chi^2 = 6.24$, $df = 2$, $p < 0.0442$; IgG, 0 %, $\chi^2 = 14.7$, $df = 2$, $p < 0.0001$, respectively, Fisher's exact test; Table 1). The sensitivity, specificity, and positive and negative predictive values of the IgE test are shown in Table 2.

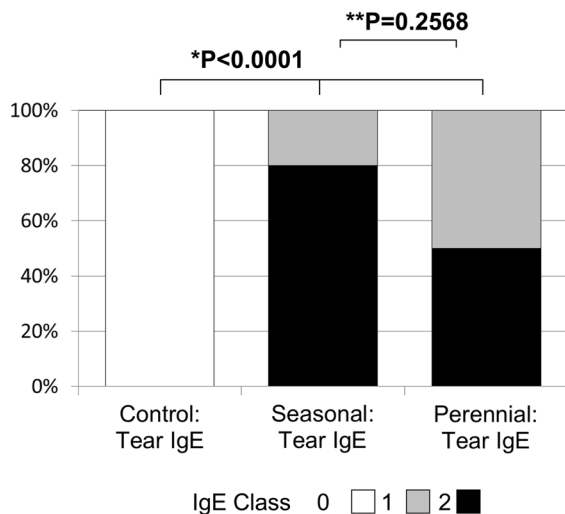


Fig. 1 Comparison of the grade of positivity for total tear immunoglobulin E (IgE) among the control, seasonal, and perennial groups. Total tear IgE was determined by the Allerwatch® test, and the total IgE score was graded as described in “Materials and methods” section. Results were compared among the three groups by Kruskal–Wallis one-way analysis of variance*, and were compared between the seasonal and perennial groups by the two-tailed Mann–Whitney *U* test**

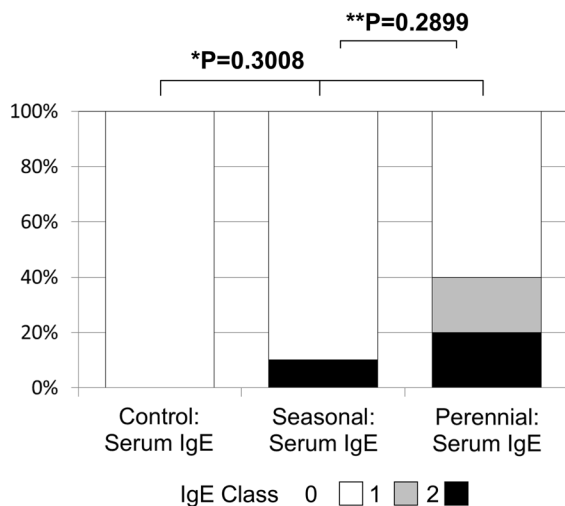


Fig. 2 Comparison of serum cat-specific IgE scores among the control, seasonal, and perennial groups. The cat-specific IgE score was determined by the ImmunoCAP Rapid® immuno-chromatography assay test, and was graded as described in “Materials and methods” section. Results were compared among the three groups by Kruskal–Wallis one-way analysis of variance*, and were compared between the seasonal and perennial groups by the two-tailed Mann–Whitney *U* test**

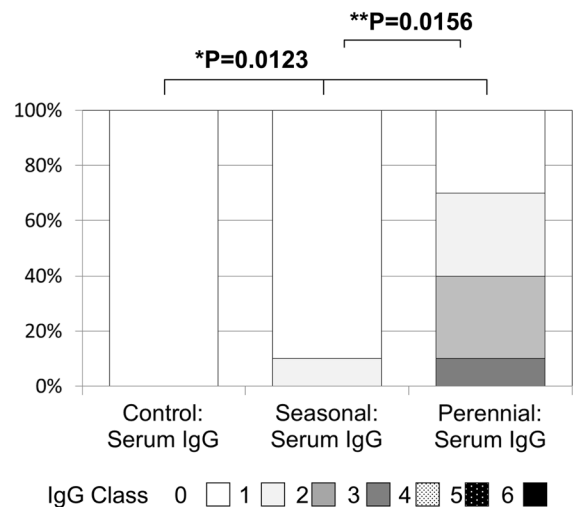


Fig. 3 Comparison of serum cat-specific IgG scores among the control, seasonal, and perennial groups. The cat-specific IgE score was determined by ELISA, and the specific IgG score was then converted into a class (0–6). Results were compared among the three groups by Kruskal–Wallis one-way analysis of variance*, and were compared between the seasonal and perennial groups by the two-tailed Mann–Whitney *U* test**

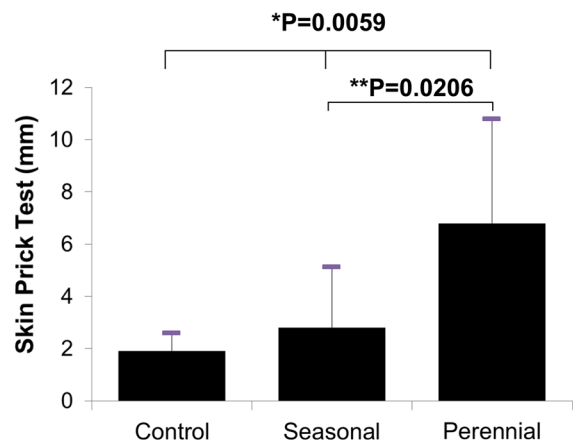
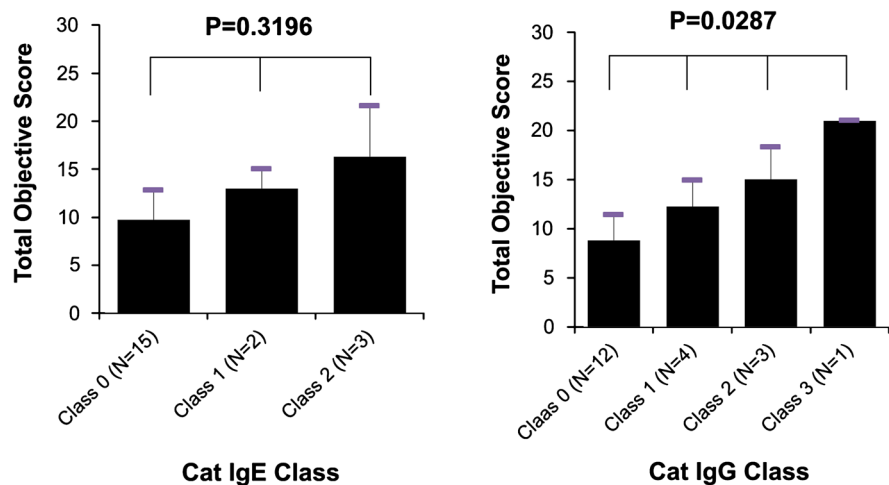


Fig. 4 Comparison of the mean wheal diameter in the skin prick test for cat allergen among the control, seasonal, and perennial groups. Results were compared among the three groups by Kruskal–Wallis one-way analysis of variance*, and were compared between the seasonal and perennial groups by the two-tailed Mann–Whitney *U* test**

The total tear IgE level and the serum cat-specific IgE and IgG levels were also compared among the control, seasonal, and perennial groups (Figs. 1, 2, 3). There were significant differences of the grade of total tear IgE (0.0 ± 0.0 vs. 1.8 ± 0.4 vs. 1.5 ± 0.5 ,

Fig. 5 Comparison of the mean total objective scores for allergic conjunctivitis among groups with different classes of serum cat-specific IgE or IgG. Patients were divided into three or four groups according to the cat-specific IgE or IgG class as described in “Materials and methods” section. Results are expressed as the mean $\pm$ SD. Comparison among the groups was done by Kruskal–Wallis one-way analysis of variance



respectively, $p < 0.00001$, Fig. 1) and the grade of serum cat-specific IgG (0.0 ± 0.0 vs. 0.1 ± 0.3 vs. 1.2 ± 1.0 , respectively, $p = 0.0123$, Fig. 3) among the three groups, but there was no significant difference in the grade of serum cat-specific IgE (0.0 ± 0.0 vs. 0.2 ± 0.6 vs. 0.6 ± 0.8 , respectively, $p = 0.3008$, Kruskal–Wallis one-way ANOVA, Fig. 2). Comparing the seasonal and perennial groups, the grade of serum cat-specific IgG was higher in the perennial group than in the seasonal group ($p = 0.0156$, Fig. 3), while there was no significant difference in the grade of total tear IgE ($p = 0.2568$, Fig. 1) or that of serum cat-specific IgE ($p = 0.2899$, two-tailed Mann–Whitney U test, Fig. 2).

The positive prevalence of SPTs for cat was higher in seasonal group (30 %) and the perennial group (70 %) than in the control group (0 %; $\chi^2 = 11.1$, $df = 2$, $p = 0.0039$, Fisher’s exact probability test, Table 1). Figure 4 shows the mean wheal diameter for cat allergen in the SPT. The mean wheal diameter was larger in the seasonal group (2.8 ± 2.3 mm) and the perennial group (6.8 ± 4.0 mm) compared with the control group (1.9 ± 0.7 mm, $p = 0.0059$, Kruskal–Wallis one-way ANOVA, Fig. 4). In addition, the mean wheal diameter was larger in the perennial group than in the seasonal group ($p = 0.0206$, two-tailed unpaired Student’s t test, Fig. 4).

Patients were divided into three groups based on the class of specific IgE for cat allergen, which were class 0 ($n = 15$), class 1 ($n = 2$), and class 2 ($n = 3$). They were also divided into four groups based on the class of specific IgG for cat allergen: class 0 ($n = 12$), class 1 ($n = 4$), class 2 ($n = 3$), and class 3 ($n = 1$), with no

Table 3 Comparison of results between patients with and without a cat at home

	Cat owner	No cat at home	<i>p</i> value
Number of patients	4/20 (20 %)	16/20 (80 %)	–
Total tear IgE			
Positive rate	4/4 (100 %)	16/16 (100 %)	–
Score (mean $\pm$ SD)	2.0 ± 0.0	1.6 ± 0.6	0.1859**
Serum cat-specific IgE			
Positive rate	1/4 (25 %)	4/16 (25 %)	0.7513*
Score (mean $\pm$ SD)	0.3 ± 0.4	0.4 ± 0.8	0.8873**
Serum cat-specific IgG			
Positive rate	3/4 (75 %)	5/16 (31 %)	0.1531*
Score (mean $\pm$ SD)	1.0 ± 0.6	0.7 ± 0.9	0.2772**
Skin prick test			
Positive rate	3/4 (75 %)	6/15 (38 %)	0.2167*
mm (mean $\pm$ SD)	5.8 ± 2.9	4.6 ± 4.0	0.4497**

Number of patients (percentage) or mean $\pm$ standard deviation (SD)

* Fisher’s exact probability test

** Two-tailed Mann–Whitney U test

patients in classes 4–6 (Fig. 5). There was a significant difference of the total objective score among the four IgG class groups ($p = 0.0287$), but no significant difference was noted among the three IgE class groups ($p = 0.3196$, Kruskal–Wallis one-way ANOVA, Fig. 5). The patients with a cat at home ($n = 4$) and those without a cat ($n = 16$) showed no significant differences with respect to positivity for tear IgE and the total tear IgE score ($p = 0.1859$ for the score), positivity for serum cat-specific IgE ($p = 0.7513$) and the IgE score ($p = 0.8873$), positivity for serum cat-specific IgG ($p = 0.1531$) and the IgG score

Table 4 Objective score for allergic conjunctivitis in IgG-positive and IgG-negative patients

	IgG-positive	IgG-negative	<i>p</i> value
Number of patients	8	12	–
Palpebral conjunctiva			
Hyperemia	2.6 ± 0.5	1.9 ± 0.7	0.0052
Edema	2.1 ± 0.9	1.4 ± 0.5	0.0120
Follicles	2.0 ± 0.9	1.2 ± 0.4	0.0202
Papillae	1.6 ± 0.7	0.8 ± 0.4	0.0038
Giant papillae	0.3 ± 0.7	0.0 ± 0.0	0.1753
Bulbar conjunctiva			
Hyperemia	2.3 ± 0.7	2.0 ± 0.5	0.0354
Chemosis	1.9 ± 1.1	1.3 ± 0.8	0.0447
Limbus			
Trantas' dots	0.5 ± 0.7	0.4 ± 0.7	0.1913
Edema	0.4 ± 0.7	0.1 ± 0.3	0.0867
Cornea			
Epithelial damage	0.0 ± 0.0	0.1 ± 0.3	0.1694
Total score	13.6 ± 4.8	9.3 ± 2.7	0.0036

The two-tailed unpaired *t* test was used to compare the clinical features of allergic conjunctivitis between patients who were positive and negative for serum cat-specific IgG. Ocular findings were assessed by slit lamp examination on a scale of 0–3 (0 = normal, 3 = severe). Then, the total objective score was calculated (0–30 points)

($p = 0.2772$), and the results of the SPT (positive rate, $p = 0.2167$; mean wheal diameter, $p = 0.4497$; Fisher's exact probability test and the two-tailed Mann–Whitney *U* test, Table 3).

To compare the clinical features of allergic conjunctivitis between patients who had a positive or negative result for serum cat-specific IgG, the 20 patients with allergic conjunctivitis were divided into an IgG-positive group ($n = 8$) and an IgG-negative group ($n = 12$, Table 4). The IgG-positive group had significantly higher scores for the clinical features of allergic conjunctivitis compared with the IgG-negative group, including the scores for palpebral conjunctival hyperemia (2.6 ± 0.5 vs. 1.9 ± 0.7 , $p = 0.0052$), edema (2.1 ± 0.9 vs. 1.4 ± 0.5 , $p = 0.0120$), follicles (2.0 ± 0.9 vs. 1.2 ± 0.4 , $p = 0.0202$), papillae (1.6 ± 0.7 vs. 0.8 ± 0.4 , $p = 0.0038$), bulbar conjunctival hyperemia (2.3 ± 0.7 vs. 2.0 ± 0.5 , $p = 0.0354$), and chemosis (1.9 ± 1.1 vs. 1.3 ± 0.8 , $p = 0.0447$), as well as a significantly higher total score (13.6 ± 4.8 vs.

9.3 ± 2.7 , $p = 0.0036$; two-tailed unpaired Student's *t* test, Fig. 3).

The mean wheal diameter for cat allergen in the SPT was strongly correlated with the total tear IgE ($r = 0.429$, $p = 0.0089$), serum cat-specific IgE ($r = 0.730$, $p = <0.0001$), and serum cat-specific IgG ($r = 0.932$, $p < 0.0001$, two-tailed Pearson's correlation coefficient analysis) in all patients (Table 5). The correlation between the mean wheal diameter and serum cat-specific IgG ($r = 0.932$, $p < 0.00001$, $n = 30$) was significantly stronger than that for serum cat-specific IgE ($r = 0.730$, $p = 0.0001$; SPT and IgG vs. SPT and IgE; $p = 0.0074$, Fisher's *z* statistics). In addition, multivariate analysis demonstrated that the mean wheal diameter for cat allergen was associated with serum cat-specific IgG (but not IgE) in all subjects [$n = 30$, odds ratio (OR) = 31.979, $p < 0.0001$; Table 5].

The total objective score was correlated with the results of all three Ig tests in all the subjects ($n = 30$, all $r > 0.500$, $p < 0.005$, Table 6). Multivariate analysis revealed that the total objective score was strongly correlated with serum cat-specific IgG ($n = 30$, OR = 23.015, $p < 0.0001$) and with total tear IgE (OR = 208.079, $p < 0.0001$, Table 6). The relation of the total objective score to the IgG and IgE scores was calculated using multivariate logistic regression models in the patients with seasonal or perennial allergic conjunctivitis ($n = 20$). An increase of the serum cat-specific IgG score by 1 point resulted in an increase of the total objective score by 3.13 points (OR = 21.893, $p < 0.0001$, Table 6). In contrast, an increase of the cat-specific IgE score by 1 point only increased the total objective score by 0.75 points, and there was no significant association between the serum cat-specific IgE score and the total objective score (OR = 0.991, $p = 0.9900$, Table 6).

Table 7 shows the correlation between the objective score for allergic conjunctivitis and serum cat-specific IgG score. The IgG score was correlated with the objective scores of palpebral and bulbar conjunctiva in all the subjects ($n = 20$, all $r > 0.40$, $p < 0.05$, two-tailed Pearson's correlation coefficients), while no correlation was found between the IgG score and corneal involvements (limbal Trantas' dots, limbal edema, or corneal epithelial damage; Table 7). Multivariate analysis revealed that no variable was independently associated with the IgG score (Table 7).

Table 5 Correlations between the skin prick test and immunoglobulin tests, as well as the results of multivariate analysis

Variables	Correlation coefficient		Multivariate analysis		
	<i>r</i>	<i>p</i> value	OR	(95 % CI)	<i>p</i> value
All subjects (<i>n</i> = 30)					
Total tear IgE	0.429	0.0089	1.148	(−0.47 to 0.74)	0.6420
Serum specific IgE	0.730	<0.0001	2.318	(−0.222 to 1.89)	0.1151
Serum specific IgG	0.932	<0.0001	31.979	(2.62 to 4.30)	<0.0001
Seasonal and perennial groups (<i>n</i> = 20)					
Total tear IgE	0.181	0.2229	0.935	(−1.62 to 1.46)	0.9277
Serum specific IgE	0.704	0.0003	2.384	(−0.45 to 2.17)	0.1815
Serum specific IgG	0.927	<0.0001	31.431	(2.40 to 4.48)	<0.0001
Seasonal group (<i>n</i> = 10)					
Total tear IgE	0.389	0.1334	2.718	(−1.00 to 2.82)	0.2666
Serum specific IgE	0.461	0.0901	7.389	(0.68 to 3.20)	0.0100
Serum specific IgG	0.749	0.0064	403.429	(3.36 to 8.41)	0.0014
Perennial group (<i>n</i> = 10)					
Total tear IgE	0.451	0.0956	0.166	(−4.04 to 0.25)	0.0977
Serum specific IgE	0.788	0.0033	0.384	(−3.19 to 1.07)	0.3335
Serum specific IgG	0.955	<0.0001	169.965	(3.14 to 6.95)	0.0007

r Two-tailed Pearson's correlation coefficients were calculated for the association between the mean wheal diameter for cat allergen in the SPT and the immunoglobulin scores, *OR* odds ratio, *CI* confidence interval

Table 6 Correlations between the total objective score and the results of immunoglobulin tests, as well as the results of multivariate analysis

Variables	Correlation coefficient		Multivariate analysis		
	<i>r</i>	<i>p</i> value	OR	(95 % CI)	<i>p</i> value
All subjects (<i>n</i> = 30)					
Total tear IgE	0.909	<0.0001	208.079	(4.69 to 5.99)	<0.0001
Serum specific IgE	0.553	0.0008	0.914	(−1.23 to 1.04)	0.8730
Serum specific IgG	0.707	<0.0001	23.015	(2.23 to 4.04)	<0.0001
Seasonal and perennial groups (<i>n</i> = 20)					
Total tear IgE	0.659	0.0008	114.825	(3.00 to 6.46)	<0.0001
Serum specific IgE	0.578	0.0038	0.991	(−1.49 to 1.45)	0.9900
Serum specific IgG	0.766	<0.0001	21.893	(1.91 to 4.24)	<0.0001
Seasonal group (<i>n</i> = 10)					
Total tear IgE	0.855	0.0008	341.495	(2.69 to 8.69)	0.0039
Serum specific IgE	−0.074	0.5809	0.400	(−2.99 to 0.98)	0.3218
Serum specific IgG	0.297	0.2021	3.211	(−2.99 to 4.95)	0.5178
Perennial group (<i>n</i> = 10)					
Total tear IgE	0.884	0.0003	1,831.677	(4.97 to 9.72)	0.0006
Serum specific IgE	0.797	0.0029	0.024	(−8.61 to 0.50)	0.1064
Serum specific IgG	0.839	0.0012	19.988	(0.85 to 4.86)	0.0158

r Two-tailed Pearson's correlation coefficients were calculated for the association between the total objective score for allergic conjunctivitis and the results of other allergic tests, *OR* odds ratio, *CI* confidence interval

Table 7 Correlations between the objective score for allergic conjunctivitis and serum cat-specific IgG score, as well as the results of multivariate analysis ($n = 20$)

Variables	Correlation coefficient		Multivariate analysis		
	<i>r</i>	<i>p</i> value	OR	(95 % CI)	<i>p</i> value
Palpebral conjunctiva					
Hyperemia	0.46	0.0424	1.366	(−0.43 to 1.06)	0.3668
Edema	0.77	<0.0001	1.213	(−0.67 to 1.06)	0.6255
Follicles	0.78	<0.0001	2.003	(−0.08 to 1.47)	0.0741
Papillae	0.68	0.0010	1.248	(−0.38 to 0.83)	0.4292
Giant papillae	0.59	0.0059	0.831	(−2.08 to 1.71)	0.8296
Bulbar conjunctiva					
Hyperemia	0.58	0.0071	2.004	(−0.16 to 1.55)	0.0998
Chemosis	0.47	0.0371	0.678	(−0.84 to 0.06)	0.0818
Limbus					
Trantas' dots	0.18	0.4562	0.710	(−1.27 to 0.59)	0.4260
Edema	0.28	0.2285	1.524	(−0.47 to 1.31)	0.3123
Cornea					
Epithelial damage	−0.16	0.4898	0.314	(−2.60 to 0.29)	0.1030

r Two-tailed Pearson's correlation coefficients were calculated for the association between the objective score for allergic conjunctivitis and serum cat-specific IgG score, *OR* odds ratio, *CI* confidence interval

Discussion

The role of IgG in allergic diseases is still unclear. The present study demonstrated that the positive rate and score for serum cat-specific IgG were both higher in the perennial group than in the control group and the seasonal group. In the perennial group, the cat-specific IgG score was more strongly correlated with the skin test reaction to cat allergen and the severity of allergic conjunctivitis (total objective score) compared with the cat-specific IgE score. Multivariate analysis also demonstrated that the cat-specific IgG score was the most important factor related to the severity of allergic conjunctivitis and the skin reaction. These results suggest that specific IgG is more closely involved in the development of perennial allergy than specific IgE.

In our preliminary study, we measured indoor allergen (cat, dog, and dust mites)-specific serum IgG in the control, seasonal, and perennial groups. The analyses showed that the cat, dog, and dust mites-specific serum IgG levels were all higher in perennial group than in seasonal group (data not shown). In the control group, specific serum IgG for cat was negative in all patients, but specific serum IgG for dog and dust mites was positive in some patients (data not shown);

therefore, we selected cat allergen, which is most suitable for analysis.

Severe allergic conjunctivitis induced by indoor allergens (house dust mites, cat, and dog) and VKC share a part of objective ocular signs such as corneal Trantas' dots, limbal edema, and giant papillae, although the severity is undoubtedly higher in VKC compared with indoor allergen-induced allergic conjunctivitis. In the current study, one patient showed mild giant papillae without corneal limbal edema or Trantas' dots, and four patients had mild Trantas' dots and limbal edema without giant papillae. These five patients were positive skin test responses to cats. Therefore, we considered these patients suffered from indoor allergen (cat)-induced allergic conjunctivitis.

The prevalence of allergic conjunctivitis associated with a positive SPT for cat allergen was 50.0 % (10/20) in our study population. Previous studies of subjects aged 20–60 have demonstrated that the SPT was positive for cat allergen in 16 % of 483 subjects in 1994 and in 26 % of 463 subjects in 2009 [29]. In addition, Aburuz et al. reported that cat allergen was the most common perennial allergen (41.6 %) among allergic rhinitis patients from Amman in Jordan based on SPT findings [30]. In Asian subjects, the SPT for

cat allergen was positive in 14.0 % (138/977) of patients with chronic rhinitis [31]. Thus, the positive rate of the SPT for cat allergen in the present study was similar to or higher than the rates reported previously (14–42 %). This was presumably because our study population was small and half of the patients had perennial allergy, which may be related to indoor allergens such as cat allergens.

Interestingly, there were no significant differences of the scores for total tear IgE, serum cat-specific IgE, and serum cat-specific IgG, as well as the skin reaction to cat allergen, between allergic patients with and without a cat in their home. There have been conflicting reports about the relationship between exposure to cat allergens and sensitization [32–34]. Almqvist et al. reported that exposure to cats increased the risk of sensitization to cat allergens, whereas exposure to dogs had no effect on sensitization to dog allergens [32]. Svanes et al. reported keeping a pet cat during childhood was associated with asthma in sensitized adults who grew up in areas with a low community prevalence of cats, while keeping a dog in childhood protected against adult allergic diseases but increased non-allergic asthma [33]. In contrast, Ritz et al. reported that children who had no regular contact with cats showed a higher prevalence rate of sensitization to cat allergens than children reporting regular contact [35]. Also, Liccardi et al. reported that patients with a cat at home had a weaker cutaneous response than patients without a cat [34]. These controversial findings probably indicate that exposure to cat allergens is necessary for sensitization, but cat ownership or regular contact with cats is not so important. Several observations support this possibility. Liccardi et al. demonstrated that even a low level of cat allergen induced a stronger cutaneous hypersensitivity response compared with direct exposure to cats [34]. Other studies have also shown that the low levels of cat allergens found in homes without cats can induce allergic sensitization and respiratory symptoms [35–37]. Even in environments without cats, such as cat-free buildings [38], public buildings [39], public transport [39, 40], and primary schools [41], passive exposure to low allergen levels could lead to sensitization. In this context, several studies have demonstrated that cat allergens are spread to various pet-free environments on the clothing of cat owners [38, 41–44].

Our second interesting finding was that IgG-positive patients had higher scores for features of allergic conjunctivitis compared with IgG-negative patients

(Table 4). There was also a significant correlation between the class of specific serum IgG for cat allergen and the total objective score (Table 5). These results indicate that the cat-specific IgG level reflects the severity of perennial allergic conjunctivitis, suggesting that the cellular mechanisms responsible for producing IgG antibodies to cat allergens may induce perennial allergic conjunctivitis. Allergens can be divided into indoor and outdoor allergens according to their source. Indoor allergens are more closely related to perennial allergic conjunctivitis, while outdoor allergens such as pollens are more frequently associated with seasonal allergic conjunctivitis [45]. Animal dander (usually from cats) is one of the major indoor allergens. Continuous exposure to indoor allergens such as cat allergens may cause chronic conjunctival proliferative changes that persist year round [46]. On the other hand, seasonal allergies are related to a wide variety of outdoor allergens such as pollens and cause acute ocular inflammation, but rarely produce the proliferative conjunctival changes that accompany chronic inflammation [46]. Thus, seasonal allergy is associated with IgE for outdoor allergens and typically occurs during the pollen season, while serum IgG levels are increased in patients with perennial allergy induced by indoor allergens. These theoretical considerations and empirical findings support the association between the class of specific IgG for cat allergens and various features of allergic conjunctivitis, as well as the total score, in patients with perennial allergy.

A limitation of the present study is that two methods were used to measure Igs, which were ELISA for IgG and immunochromatography for IgE. In our clinic, we use immunochromatography to measure IgE as a rapid point-of-care assay that provides a result within 20 min, while measurement of IgE by standard ELISA takes much longer. So far, an immunochromatography assay kit for the detection of IgG has not become commercially available, but we hope for the development of such a kit in the near future. Second limitation is that the relationship between the severity of cat allergen-induced allergic conjunctivitis and cat-specific IgG levels in Japanese populations may not be applicable to European populations.

In conclusion, the present study demonstrated that the serum level of cat-specific IgG was more closely associated with the clinical features of perennial allergic conjunctivitis than the cat-specific IgE level. This suggests that measurement of allergen-specific IgG

antibodies may be more helpful for detecting the allergens responsible for perennial allergic diseases than the current method of testing for allergen-specific IgE.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Health Labour Sciences Research Grant from The Ministry of Health Labour and Welfare of Japan.

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

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