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Original Article

Evaluation of FcεRI-binding serum IgE in patients with ocular allergic diseases

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Abstract

We evaluated high-affinity receptor for IgE (FcεRI)-binding serum IgE in patients with atopic keratoconjunctivitis (AKC; $n = 31$) and with seasonal allergic conjunctivitis (SAC; $n = 13$) by enzyme-linked immunosorbent assay (ELISA) using a recombinant soluble form of the human FcεRIα ectodomain (soluble α). The quantities of FcεRI-binding IgE are compared with those of total IgE measured by a conventional sandwich ELISA. Both of the quantities of FcεRI-binding and total IgE in AKC were significantly larger than those in SAC ($P < 0.001$). In contrast, the proportion of FcεRI-binding IgE (FcεRI-binding IgE/total IgE; %) in SAC was significantly larger than that in AKC ($P < 0.001$), although significant reverse correlation was observed between the proportion of FcεRI-binding IgE and total IgE in both AKC and SAC. Significantly, a higher proportion of FcεRI-binding IgE in SAC than that in AKC may reflect the differences in pathologic states of AKC and SAC that are caused by a disparity in immune responses in these diseases.

Key words: atopic keratoconjunctivitis, ELISA, FcεRI, FcεRI-binding IgE, seasonal allergic conjunctivitis.

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Introduction

The high-affinity receptor for IgE, FcεRI, is expressed on mast cells and basophils. Cross-linking of IgE on the receptors by multivalent antigens activates these cells and induces cellular degranulation, resulting in allergic inflammation. In patients with allergic diseases, serum IgE values are generally increased compared with those in normal subjects. Among all IgE molecules in the serum, IgE that actually induce an allergic reaction should be able to bind to the receptor. Therefore, it is of great importance to estimate the quantities of serum IgE actually able to bind to FcεRI to evaluate clinical conditions of patients with allergic diseases.

FcεRI has a tetrameric structure composed of α-, β- and γ2-chains, of which the α-chain binds IgE with high affinity.[1–3] Recently, Ra and colleagues developed a novel enzyme-linked immunosorbent assay (ELISA) system to detect FcεRI-binding IgE by using a recombinant soluble form of the human FcεRIα ectodomain (soluble α).[4] In the present study, we measured serum FcεRI-binding IgE in patients with atopic keratoconjunctivitis and seasonal conjunctivitis by this newly developed ELISA (soluble-α ELISA) and compared the quantities of FcεRI-binding IgE with those of total IgE measured by a conventional sandwich ELISA.

Methods

Sera

Sera were obtained from 31 patients with atopic keratoconjunctivitis (AKC; 15 men and 16 women; mean ±SD, 21.4 ± 6.3 years old) and 13 patients with seasonal allergic conjunctivitis (SAC; 6 men and 7 women; 39.8 ± 17.3 years old). The study followed the tenets of the Declaration of Helsinki.

Enzyme-linked immunosorbent assay to detect FcεRI-binding IgE using soluble α

Microtiter® Plates (Immulon 2; Dynatech Laboratories, Chantilly, VA, USA) were coated with 180 ng soluble α produced by CHO transfectants[4–6] in 90 μL 50 mmol/L carbonate buffer (pH 9.4) overnight at 4°C. Each well was washed five times with phosphate-buffered saline (PBS), pH 7.2, containing 0.05% Tween 20 (Wako Pure Chemical Industries Ltd, Osaka, Japan; washing buffer), filled with 200 μL PBS containing 1% casein (skimmed milk; Snow-Brand Industries Co. Ltd, Sapporo, Japan) and 0.02% NaN_3 and incubated for 1 h at 37°C. Plates were washed and 100 μL human IgE, purified from the serum of an IgE myeloma patient, or sera diluted with PBS containing 0.1% casein was added to each well and the plates were incubated for 3 h at 37°C. Each well was washed, filled with 100 mL horseradish peroxidase (HRP)-labeled goat antihuman IgE (Organon Teknica Corp., Durham, NC, USA) (2.32 μg/mL in PBS containing 0.1% casein) and plates were incubated for 1 h at 37°C. After washing, 100 μL o-phenylenediamine (OPD; Wako Pure Chemical Industries Ltd; 2 mg/mL in citrate phosphate buffer pH 5.0, containing 0.015% H_2O_2) was added to each well and the plates were incubated at room temperature in the dark for 15 min. The enzyme reaction was stopped by adding 100 μL 2 mol/L H_2SO_4 to each well. Optical absorbance at 490 nm was read using Bio-Rad® microplate reader (model 450; Bio-Rad Laboratories Inc., Hercules, CA, USA).

Enzyme-linked immunosorbent assay to detect total IgE by the sandwich method

To evaluate total IgE, ELSIA®-IgE kits (International Reagents Corp., Kobe, Japan) were adopted. The plates were coated with a monoclonal mouse antihuman IgE antibody, filled with standard IgE or diluted sera, incubated for 1 h at room temperature and washed five times with washing solution. Peroxidase (POD)-labeled goat antihuman IgE antibody was added to each well and the plates were incubated for 30 min at room temperature, washed and then incubated with OPD in substrate solution for 30 min in the dark at room temperature. The reaction was stopped by adding 2 mol/L H_2SO_4 to each well and the optical absorbance was read as described earlier.

Statistical analysis

Data were statistically analyzed by the Mann–Whitney *U*-test and Fisher's Z-transformation using STATVIEW® (Abacus Concepts, Inc., Berkeley, CA, USA). A probability of 5% or less was considered statistically significant.

RESULTS

Comparison of FcεRI-binding and total IgE levels in the sera of AKC and SAC patients

The values of FcεRI-binding serum IgE detected by the newly developed soluble-α ELISA were compared with those of total IgE detected by a conventional sandwich ELISA (Table 1). The values of FcεRI-binding IgE were substantially lower than those of total IgE in both AKC and SAC sera, suggesting that all of the serum IgE could not necessarily bind to the FcεRI. The quantity of FcεRI-binding IgE in AKC patients (563 ± 341 ng/mL) was significantly larger than that in SAC (222 ± 232 ng/mL, $P < 0.001$). A significant difference was also observed between the quantity of total IgE in AKC (4685 ± 6739 ng/mL) and that in SAC (418 ± 570 ng/mL, $P < 0.0001$). In remarkable contrast, the proportion of FcεRI-binding IgE was significantly larger in SAC (73.3 ± 24.7%) than in AKC (31.9 ± 24.3%, $P < 0.0001$), as shown in Fig. 1. When we plotted the FcεRI-binding IgE against total IgE (Fig. 2), a correlation was observed between these values in both AKC ($r = 0.770$, $P < 0.0001$) and SAC ($r = 0.950$, $P < 0.0001$).

The proportion of FcεRI-binding IgE in AKC and SAC sera

A reverse correlation was observed in the plot of FcεRI-binding IgE/total IgE (%) against total IgE (ng/mL) in both AKC ($r = -0.588$, $P < 0.0005$) and SAC ($r = -0.741$,

Table 1 Comparison of FcεRI-binding and total IgE levels in the sera of AKC and SAC patients

	AKC	SAC
Total IgE (ng/mL)	4685 ± 6739*	418 ± 570
FcεRI-binding IgE (ng/mL)	563 ± 341†	222 ± 232
FcεRI-binding IgE/total IgE (%)	31.9 ± 24.3*	73.7 ± 24.7

Data are expressed as the mean ± SD. *$P < 0.0001$, †$P < 0.001$ compared with seasonal allergic conjunctivitis (SAC) patients. AKC, atopic keratoconjunctivitis.

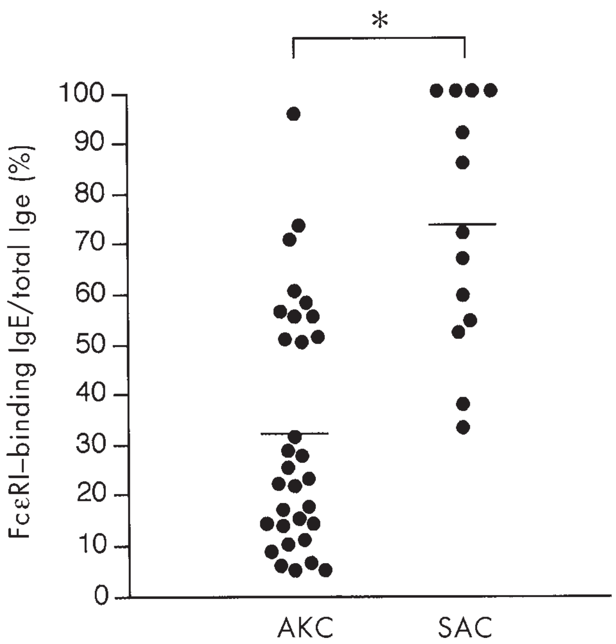

Fig. 1 Comparison of the proportion of FcεRI-binding serum IgE in atopic keratoconjunctivitis (AKC) and seasonal allergic conjunctivitis (SAC). Serum IgE that bound to the recombinant soluble human FcεRIα ectodomain, coated on the plates, was detected by a polyclonal antihuman IgE antibody (FcεRI-binding IgE). Serum total IgE was evaluated by a conventional sandwich enzyme-linked immunosorbent assay. The proportion of FcεRI-binding IgE in SAC (73.7 ± 24.7%) was significantly larger than that in AKC (31.9 ± 24.3%). *$P < 0.0001$, (–), mean.

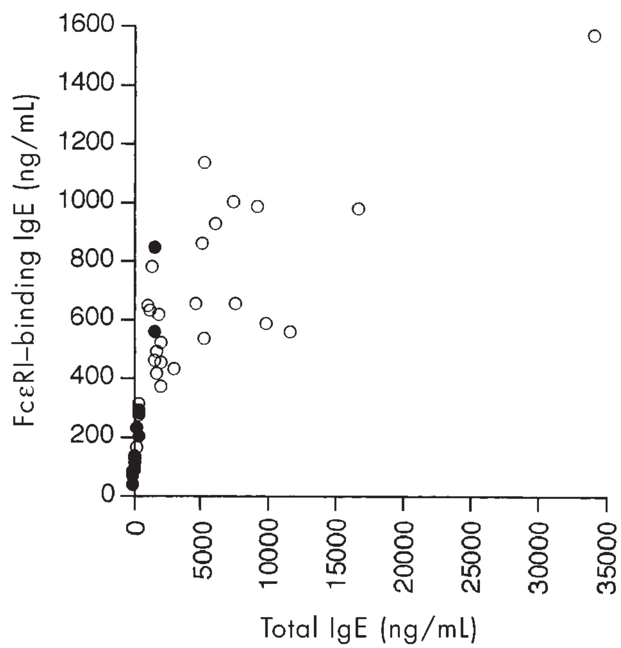

Fig. 2 Correlation between the quantities of FcεRI-binding and total IgE. A positive correlation was observed both in atopic keratoconjunctivitis (○; $n = 31$, $r = 0.770$, $P < 0.0001$, $y = 0.039x + 380.7$) and in seasonal allergic conjunctivitis (●; $n = 13$, $r = 0.950$, $P < 0.0001$, $y = 0.38x + 60.5$).

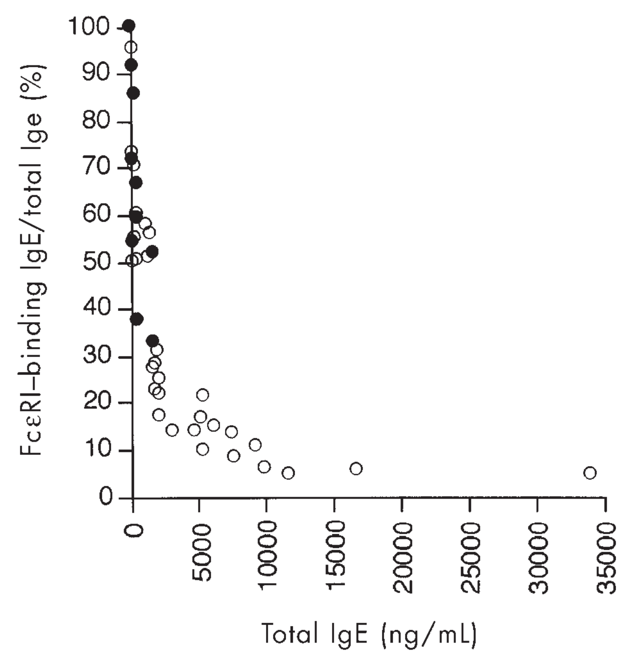

Fig. 3 Correlation between the quantity of total IgE and the proportion of FcεRI-binding IgE. A negative correlation was observed both in atopic keratoconjunctivitis (○; $n = 31$, $r = -0.588$, $P < 0.0005$; $y = -0.0021x + 41.9$) and in seasonal allergic conjunctivitis (●; $n = 13$, $r = -0.741$, $P < 0.005$, $y = -0.032x + 86.6$).

$P < 0.005$), as shown in Fig. 3. Particularly in hyper-IgE sera (IgE > 5000 ng/mL) from AKC patients, the proportion of FcεRI-binding IgE was below 20%.

Discussion

It is well known that IgE plays a crucial role in allergic reactions by activating mast cells and basophils to release a variety of inflammatory mediators and cytokines. When FcεRI-bound IgE on mast cells are cross-linked with multivalent antigens, a series of biochemical cascades are triggered toward cellular activation. In addition, there have been several papers recently published on the expression and function of FcεRI in other kinds of cells. Langerhans cells in the skin,[7–9] some populations of eosinophils[10,11] and monocytes[12] also express FcεRI on the cell surface and are activated by cross-linking of FcεRI. Therefore, it is important to evaluate functional IgE that actually binds to FcεRI for a further insight into allergic states.

Soon after the discovery of IgE, Ishizaka and Ishizaka reported that the quantity of cell-binding IgE did not necessarily correlate with total serum IgE levels in atopic individuals.[13] Subsequently, there were several reports published that suggested that serum IgE may be heterogeneous with regards to cell-binding and histamine-releasing properties in the passive cutaneous anaphylaxis (PCA) reaction.[14–17] Although a variety of ELISA systems are widely adopted to evaluate serum IgE levels, as one of the useful means for clinical diagnosis of allergy, they are not concerned with the ability of IgE to bind to the receptor.

We have developed a novel ELISA system to detect FcεRI-binding IgE (soluble-α ELISA) and have evaluated FcεRI-binding serum IgE in patients with AKC and SAC. The quantity of FcεRI-binding serum IgE correlated with the serum total IgE level, both in AKC and SAC (Fig. 2). In a remarkable contrast, when we plotted the proportion of FcεRI-binding IgE against total IgE, a reverse correlation was observed in both of the two groups (Fig. 3) as observed in patients with atopic dermatitis, bronchial asthma and in normal controls.[4] Particularly in hyper-IgE sera from AKC patients (> 5000 ng/mL), the proportion of FcεRI-binding IgE was less than 20% (Fig. 3) and there was a significant difference in this value between the two groups (Fig. 1). Atopic keratoconjunctivitis is a chronic keratoconjunctivitis often observed with atopic dermatitis and SAC is an acute conjunctivitis repeated in the period of pollen season. Both FcεRI-binding and total IgE were significantly greater in AKC than in SAC patients; however, the proportion of FcεRI-binding IgE was significantly higher in SAC than in AKC patients (Table 1). There appears to be two subgroups of the AKC patients, based on the proportion of FcεRI-binding IgE. One group of 11 patients has more than 40% of FcεRI-binding IgE and the other has less than 40%. However, there is no significant difference in the severity of corneal complications or in the incidence of cataract and retinal detachment between these two groups. These results may reflect the difference of allergic states in these diseases, namely chronic and acute allergic states. In a chronic allergic state, such as AKC, the absolute quantities of FcεRI-binding and total IgE were increased, but the proportion of FcεRI-binding IgE was decreased. This suggests that in hyper-IgE sera there may be some serum factors that interfere with IgE binding to FcεRI[18–26] and/or IgE that is structurally unable to be bound, due to carbohydrate modification and/or crucial changes of the primary sequence in the Fcε chain. Whatever these factors may be, our results indicate that the proportion of FcεRI-binding IgE is higher in acute allergic diseases, such as SAC, and the evaluation of FcεRI-binding IgE fraction may contribute to a distinction between IgE-dependent acute allergic diseases and chronic allergic diseases.

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