

IgE antibody-specific activity in human allergic disease

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Abstract IgE antibody concentration, affinity, clonality and specific activity (also known as the allergen-specific IgE to total IgE ratio) influence the translation of IgE responses into clinically evident allergic symptoms following allergen exposure. Reported IgE-specific activity levels $>3\text{--}4\%$ place allergic individuals undergoing anti-IgE (Omalizumab[®]) therapy at a disadvantage for poor resolution of their allergy symptoms following manufacturer's recommended dosing schemes. We investigated the hypothesis that the specific activity of the IgE antibody response is highly variable with respect to age, allergen specificity and an individual's total serum IgE level. Second, we investigated whether the IgE-specific activity level influences the extent and rate of loss of effector cell mediator release. IgE-specific activity distributions were plotted against age, allergen specificity and total serum IgE using 18,950 paired total IgE and allergen-specific IgE antibody data obtained from the analysis of sera from 3,614 allergic subjects and covering 182 allergen specificities. The fraction of specific IgE antibody of the total serum IgE was dependent on age of the individual, epitope specificity (clonality) and total serum IgE. The youngest group of allergic individuals with the lowest total serum IgE levels tended to have the highest allergen-specific IgE to total IgE ratios. Hymenoptera venom (54%), peanut (33%) and milk (27%) were the three allergen specificities that elicited the highest frequency of IgE-specific activities $>4\%$ among sensitized individuals. A prospective double-blind, placebo-controlled clinical study involving anti-IgE treatment of cat-allergic subjects showed IgE-specific activity was remarkably constant over the 16-week course of

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treatment, despite the up to 8-fold rise in total serum IgE following repetitive Omalizumab® administration. Changes in specific and total IgE levels paralleled each other in patients receiving anti-IgE therapy. The fastest rate of reduction in cat allergen-induced basophil histamine release following anti-IgE therapy was observed when the cat-specific IgE to total IgE ratio was <2.5%. This reflected the more rapid loss of surface cat-specific IgE antibody with anti-IgE therapy in allergic individuals who displayed a more diverse IgE antibody repertoire. We conclude that IgE-specific activity is an age-, IgE heterogeneity- and total serum IgE-dependent variable that influences the magnitude of effector cell mediator release, and by inference, ultimate allergic symptom induction.

Keywords Allergy · Human · IgE · Concentration · Specific activity · Specific IgE to total IgE ratio · Clonality · Affinity

Introduction

In 1967, IgE was identified as the immunoglobulin isotype, structurally unique from IgG, IgA, IgM and IgD, that serves humans as the immunologic gatekeeper of the type 1 or immediate hypersensitivity response [1, 2]. Over more than 40 years, clinical immunology laboratories have been supporting the clinician in the diagnosis of IgE-mediated disorders by quantifying the level of total and allergen-specific IgE in serum. The relative clinical importance of the total level of circulating IgE and quantification of the portion of IgE that is allergen-specific has oscillated over the years. Total serum IgE, for instance, has been viewed by oncologists as a useful marker of B lymphocyte reconstitution in bone marrow transplant patients [3]. In atopic individuals, elevated total serum IgE levels have also been associated with a predisposition for airway hyper-responsiveness and asthma [4, 5]. Detection of allergen-specific IgE has been generally considered the more clinically useful analyte, since it is a specific biomarker for the atopic state which allows linkage of symptoms to particular allergen exposure. Detection of allergen-specific IgE antibodies in serum has been used clinically to confirm sensitization to a particular allergen in support of a history-based diagnosis of allergic forms of rhinitis, asthma, dermatitis, urticaria, angioedema, ocular inflammation, anaphylaxis and food and insect sting allergy [6].

In recent years, IgE antibody assays have matured with the availability of technologically advanced autoanalyzers that display remarkable performance in terms of their analytical sensitivity, linearity and precision [6, 7]. However, in spite of the use of a common calibration system and sophisticated automation, the different clinically used allergen-specific IgE antibody assays continue to detect different populations of IgE antibody in serum [7]. Thus, IgE antibody results generated by any of the three principal IgE antibody autoanalyzers (ImmunoCAP-Phadia; Immulite-Siemens; HYTEC-Hycor-Agilent) used in clinical laboratories are considered comparable but not interchangeable. This emphasizes the need for the clinician to know the method that was used to generate a patient's specific IgE results, since all three methods report using the same units (kUa/L).

From the time IgE was discovered, it has been known that specific IgE antibody can be detected in the skin or blood of a sensitized individual in the absence of any clinically evident allergic reactions, despite known allergen exposure. Reasons for this apparent lack of agreement between the presence of detectable IgE antibody in the skin and blood and the slower emergence of clinically evident allergic symptoms have not been fully

elucidated. One contribution factor relates to the nature of the humoral immune response, that when combined with variations associated with the allergen, patient and assay reagents and technique, contribute to this apparent inconsistency between IgE antibody presence in serum and skin and the absence of clinically evident allergic symptoms.

In 2008, investigators confirmed four (IgE) humoral immune response parameters that alter mast cell and basophil mediator release in an allergic patient [8]. These four humoral response parameters are believed to be operationally important in translating a state of sensitization (IgE antibody positivity) into a state of clinically manifested allergic symptoms. The allergen-specific IgE antibody's concentration, affinity (tightness of binding), clonality (epitope specificity) and specific activity (alternatively referred to as the allergen-specific IgE to total IgE ratio) each was shown to impact on the extent of effector cell activation. The investigators used a panel of 10 mouse/human chimeric IgE antibodies with differing affinities and epitope specificities. They also generated nine variants of human *Dermatophagoides pteronyssinus* group 2 allergen (Derp2)-specific recombinant IgE antibodies that were cloned from a dust mite allergic patient's B cells using a phage display technique. A flow cytometric-based basophil activation assay was performed using combinations of IgE antibody affinities ranging from 0.0358 to 291 nM, with up to 9 distinct Derp2 binding epitopes. Important to the present discussion, the Derp2-specific IgE to total IgE ratios were adjusted by adding varying amounts of non-Derp2-specific IgE before they were added to lactic acid treated basophils for passive sensitization. The study concluded that higher levels of basophil activation could be elicited with higher overall serum Derp2-specific IgE antibody concentrations, broader clonality or recognition of Derp2 epitopes and more highly matured IgE antibody affinities and higher Derp2-specific IgE to total IgE ratios or specific activities. The ability to induce more rigorous basophil activation responses has been associated with the more marked expression of allergic symptoms.

Currently available serological assays provide excellent characterization of the IgE antibody concentration and the fraction of the total IgE that is specific for a particular allergen. Three United States Food and Drug Administration (US FDA)-cleared autoanalyzers quantitatively measure the concentration of total IgE and allergen-specific IgE antibody in serum to an analytical sensitivity of 2 and 0.1 kUa/L, respectively, where 1 Unit = 2.4 nanograms of IgE [7, 9]. Newer chip-based fluorescent microarray systems permit elegant mapping of primary protein structure epitope specificities using overlapping peptide matrices [9]. IgE antibody affinity determinations can be assessed using surface Plasmon resonance technology (e.g., Biacore technology). However, from a practical point of view, these latter two analytical measurements are not available in clinical immunology laboratories for routine evaluation of patient sera. Affinity determinations and epitope mapping of IgE responses are thus considered principally research investigations. The remainder of this paper will focus on a discussion of the importance of the fourth humoral immune response parameter, namely, specific activity of the IgE antibody response. The diagnostic and clinical importance of the IgE-specific activity is less intuitive and well studied than the diagnostic importance of IgE antibody concentration, affinity and allergen specificity.

IgE antibody response-specific activity

Of the four modes of allergic disease management (avoidance, pharmacotherapy, immunotherapy and anti-IgE therapy), the latter has become important to the understanding of

clinical importance of the IgE antibody's specific activity. Treatment with anti-IgE (Omalizumab[®], trade name Xolair[®]) was approved by the U.S.-FDA in 2003 for the treatment of severe IgE-mediated asthma [10]. The dose of Omalizumab[®] that is administered to the patient is computed based on the patient's total serum IgE concentration and body weight. It should in theory be administered in molar excess to circulating IgE levels in order to reduce the so-called free IgE level in circulation. "Free" IgE constitutes IgE molecules that circulate uncomplexed with Omalizumab[®] and thus are able to arm or sensitize mast cells, basophils and dendritic cells by binding onto the high affinity IgE receptor.

In 2009, Johansson et al. [11] tested the hypothesis that the manufacturer's recommended doses of Omalizumab[®] were not effective in patients with high allergen-specific to total IgE antibody ratios. Cat-allergic subjects were recruited for a double-blind, placebo-controlled trial with symptoms of rhino-conjunctivitis and/or asthma associated with cat allergen exposure. All had IgE antibodies specific for cat dander in different proportions in relation to their total serum IgE. They were excluded if they had had prior cat allergen immunotherapy. Total and cat-specific IgE antibody levels were measured by ImmunoCAP (Phadia, Uppsala Sweden) prior to Omalizumab[®] administration. Subjects were then divided into two groups, one with a low cat-specific activities ($\leq 1\%$ cat-specific IgE to total IgE ratio) and the other with a high ($\geq 3.8\%$) IgE-specific activities. Placebo subjects in each group received a sham injection, and test subjects were injected with Omalizumab[®] using manufacturer's recommended doses for 16 weeks.

Blood was collected at the beginning and end of treatment for analysis by flow cytometry to determine the extent of basophil activation as a function of the concentration of added cat allergen. The basophil allergen threshold sensitivity, called CDsens, was measured as the inverted value of the allergen concentration producing 50% of maximum CD63 up-regulation on basophils multiplied by 100. The magnitude of the disease-relevant IgE antibody fraction that was cat allergen-specific of the total IgE was shown to predict the efficacy of anti-IgE treatment in terms of ablating activation when subject basophils were incubated with varying concentrations of cat dander allergen. No change in basophil CD-sens was detected in the groups that received placebo injections when baseline responses were compared to those at the end of treatment. However, for Omalizumab[®]-treated patients at the end of the trial, there was a significant difference in CD-sens between the high group (cat-specific IgE fraction $> 3.8\%$) where none of the patients lost their basophil sensitivity to cat dander extract and the low group (cat-specific IgE fraction $< 1\%$), where 13 of 18 subjects' basophils became negative to surface marker evidence of activation in vitro following cat dander allergen stimulation.

The study concluded that the currently recommended doses of Omalizumab[®] can efficiently eliminate IgE antibodies to a level that reduces allergic symptoms when the fraction of cat-specific to total IgE in the patient is less than 1%. However, the Omalizumab[®] dose recommended by the manufacturer was considered insufficient to produce loss of basophil sensitivity, the chosen indicator of anti-IgE treatment efficacy, in subjects with a cat-specific to total IgE fraction greater than 4%. So, an IgE-specific activity of 4% or greater, was suggested as a biomarker for an IgE antibody response as makes clinical symptom improvement more difficult to accomplish with anti-IgE therapy. The higher the specific activity of a particular IgE antibody specificity, the higher the probability that there will be a sufficient surface density of IgE molecules of the same specificity to induce mast cell and basophil activation when allergen is encountered.

Distribution of the specific to total IgE fraction in allergy patients

Clinical immunology laboratories measure IgE antibody in human serum to over 200 allergen specificities. Of these, only a small number are known to cause severe life-threatening allergic symptoms in the sensitized patient. The route of exposure can involve ingestion (e.g., peanut) and injection (drugs [insulin, penicillin], Hymenoptera venom). The majority of allergens, however, are known to induce morbidity with chronic symptoms such as rhinitis, pruritis, dermatitis and urticaria. We investigated the hypothesis that the fraction of the total IgE that is allergen specific may vary as a function of the allergen specificity and thus in principal contribute to the greater or lesser severity of allergic symptoms. If peanut allergen were to induce a higher specific activity than Timothy grass pollen, this could in part explain why allergic symptoms associated with the ingestion of peanut could produce more severe allergic symptoms than the inhalation of Timothy grass pollen. We chose to investigate IgE-specific activity, because it has been the least studied humoral immune response parameter. Little is known about the relative IgE-specific activity distribution among allergy patients of different ages and whether it varies with allergen specificity, more specifically between aeroallergens and food allergens.

Over years, the Johns Hopkins Dermatology, Allergy and Clinical Immunology Reference Laboratory has measured total IgE and specific IgE by ImmunoCAP (Phadia, Uppsala, Sweden) to ~200 allergen specificities in the sera of thousands of atopic individuals who presented with allergic symptoms and were worked up with consent for possible allergic sensitization. A subset of these data on which age was available have been examined to study the effect of age and allergen specificity on the distribution of IgE-specific activity. The specific activity for each total IgE and allergen-specific IgE measurement pair was computed by dividing the total serum IgE level in kU/L into the allergen-specific IgE level in kUa/L and reporting it as percent. In the ImmunoCAP, 1 kUa/L of allergen-specific IgE has been shown to be equivalent to 1 kU/L of total serum IgE [12].

Effect of age on IgE-specific activity

Figure 1 displays the age-related distribution of 18,950 IgE-specific activities that were obtained by analysis of 3,614 sera from atopic individuals who ranged in age from 0.23 to 88.8 years. This study population was heavily skewed toward the younger age groups, since children are more commonly evaluated serologically for specific IgE to foods and aeroallergens as they undergo the “atopic march” from food allergy and atopic eczema to upper and lower respiratory allergic disease [13]. The concentration of IgE in serum is known to age-dependent and thus total serum IgE levels are evaluated in relationship to age-adjusted non-atopic population data when they are used for diagnostic decisions [14]. The study dataset was subdivided into quartiles based on age: 1st quartile: 0.23–3.7 years; 2nd quartile: 3.8–6.4 years, 3rd quartile: 6.5–10.5 years and 4th quartile: 10.6–88.8 years. A progressive decrease in the mean IgE-specific activity was observed from 0.23 to ~10 years of age. No significant further change in the IgE-specific activity was observed between the third and fourth age quartiles. Since the concentration of total and specific IgE are commonly lowest in the youngest patients, this suggests that young people as a general rule will tend to have a higher fraction of their total IgE that is specific for a particular allergen specificity than older allergy patients. There are of course exceptions to this rule.

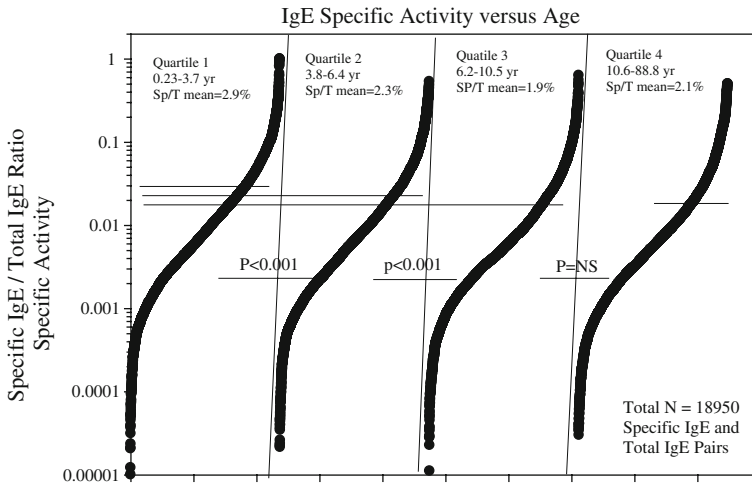


Fig. 1 Sera from sequential 3,614 atopic individuals were analyzed for total and allergen-specific IgE by ImmunoCAP (Phadia). A total of 18,950 specific IgE and total IgE paired data were used to construct this figure, which was subdivided in quartiles based on age (0.23–3.7 years; 3.8–6.4 years; 6.5–10.5 years and 10.6–88.8 years). IgE antibody was measured to a total of 182 allergen specificities including dust mite, pet epidermals, molds, foods, Hymenoptera venoms, occupational allergens and pollens from trees, grasses and weeds. The mean IgE-specific activity for this study population decreased significantly from 0.23 years to an age of 10.5 years, at which time it appeared to not change further. This trend in the IgE-specific activity coincides with the well-established increase in total serum IgE levels from birth to the age of 10–15 years

Effect of allergen specificity on the IgE-specific activity distribution

Total and allergen-specific IgE pairs were measured to 182 different allergen specificities covering common indoor aeroallergens (dust mite, pet and rodent epidermal allergens, cockroach), food allergens (nuts, legumes, chicken egg, cow's milk, shellfish, fish, grains) and outdoor aeroallergens (grass, tree and weed pollens, molds). Figure 2 displays the IgE-specific activity distributions for representative indoor aeroallergens (Fig. 2a), food allergens (Fig. 2b), outdoor aeroallergens (Fig. 2c) and Hymenoptera venom (Fig. 2d). The number of subjects in each specificity distribution and the percentage of these subjects who had an IgE-specific activity $>4\%$ are noted in the figure. The percentage of the population studied that had IgE-specific activities greater than a previously reported target of 4% [11] ranged from 3.5% for the mold *Aspergillus fumigatus* to a high of 54% for Hymenoptera venom sensitive patients. For most allergens, less than one-fourth of patients had IgE-specific activities greater than the preestablished target of 4%. Milk, peanut and Hymenoptera venom were exceptional for their higher percentage of overall subjects with an IgE-specific activity $>4\%$ (27, 33, 54%, respectively). An IgE-specific activity $>4\%$ can place the allergic patient at a disadvantage for resolving allergic symptoms if they undergo anti-IgE therapy with the recommended dosing schedule [11]. This prediction relies on the assumption that the degree of basophil reactivity reduction is predictive of clinical symptom occurrence.

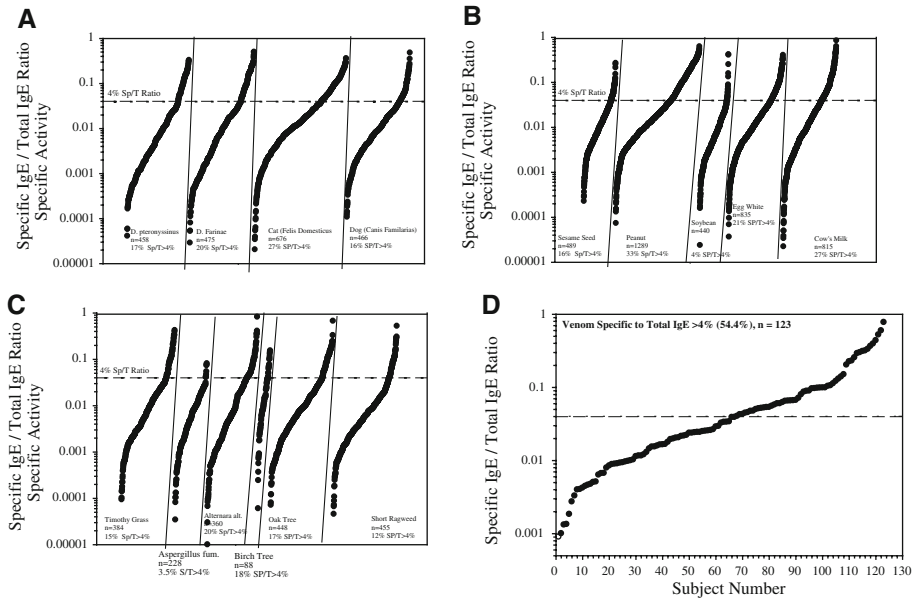


Fig. 2 **a** Distribution of IgE-specific activities for 4 common indoor aeroallergens: dust mite: *Dermatophagoides pteronyssinus* ($n = 458$), dust mite *Dermatophagoides farinae* ($n = 475$), cat dander ($n = 676$) and dog dander ($n = 466$). IgE-specific activities for these 4 allergen specificities spanned 4 log concentrations (range: 0.02–49%). The percentage of these subjects who displayed IgE-specific activities greater than an arbitrary target threshold of 4% ranged from 16% (dog dander) to 27% (cat dander). These data are not controlled for subject age. **b** Distribution of IgE-specific activities for 5 important food allergens: sesame seed ($n = 489$), peanut ($n = 1,289$), soybean ($n = 440$), chicken egg white ($n = 835$), and cow's milk ($n = 815$). IgE-specific activities for these 5 allergen specificities spanned 4 log concentrations (range: 0.02–49%). The percentage of these subjects who displayed IgE-specific activities greater than an arbitrary target threshold of 4% ranged from 4% (soybean) to 33% (peanut). These data are not controlled for subject age. **c** Distribution of IgE-specific activities for 6 representative outdoor allergens: Timothy grass ($n = 384$), mold *Aspergillus fumigatus* ($n = 228$), mold *Alternaria alternata* ($n = 360$), birch tree pollen ($n = 88$), oak tree pollen ($n = 448$), short ragweed ($n = 455$). IgE-specific activities for these 6 allergen specificities spanned 4 log concentrations (range: 0.01–82%). The percentage of these subjects who displayed IgE-specific activities greater than an arbitrary target threshold of 4% ranged from 3.5% (*Aspergillus fumigatus*) to 20% (*Alternaria alternata*). These data are not controlled for subject age. **d** Distribution of IgE-specific activities for Hymenoptera venom sensitive patients. The figure combines data from subjects with positive IgE antibody serology to venoms from yellow jacket, honeybee, white faced hornet, yellow hornet and Polistes wasp ($n = 123$). The data are sorted in order of increasing specific IgE to total IgE ratios. The IgE-specific activities for Hymenoptera patients spanned 3 log concentrations (range: 0.1–78%). Interestingly, 54% Hymenoptera venom subjects displayed an IgE-specific activity greater than 4%. These data are not controlled for subject age

IgE-specific activity as a function of total serum IgE

To be an candidate for anti-IgE therapy with Omalizumab[®], one must have a total serum IgE between 30 and 700 kU/L. Greater efficacy of Omalizumab[®] treatment has been reported in patients who have total IgE levels above 75 kU/L when they were compared to placebo [15]. Moreover, the fraction of the total IgE that is specific for a given allergen specificity reportedly increases as the total serum IgE level decreases [16]. To confirm the observations in these initial reports, we investigated this directly by plotting the specific IgE to total IgE ratio in a given serum as a function of the total serum IgE in the same

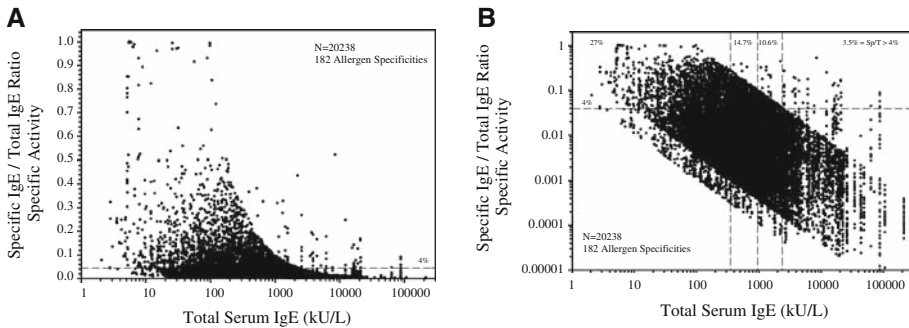


Fig. 3 **a, b** IgE-specific activity (allergen-specific IgE to total IgE ratio) for 20,238 total IgE:allergen-specific IgE pairs involving 182 allergen specificities. **a** presents the ordinate at a linear variable while **b** displays the same data as the common log of the IgE-specific activity. **a** shows that the predominant total serum IgE levels range from 20 to 5,000 kU/L, while **b** shows that the probability of obtaining a high IgE-specific activity (e.g., >4%) increases with decreasing total serum IgE concentration

serum (Fig. 3). The percentage of individuals who displayed specific IgE to total IgE ratios above the targeted 4% decreased as the total serum IgE increased (1° quartile 27% [total IgE 2.1–209 kU/L], 2° quartile: 14.7% [total IgE 210–512 kU/L], 3° quartile 10.6% [total IgE 513–1,286 kU/L], 4° quartile 3.5% [1,287–2,16,610 kU/L]). Of the subjects with total serum IgE levels between 2.9 and 30 kU/L or less, 64% of the measurements had IgE-specific activities >4%. These data support the conclusion that allergen-specific IgE diversity is restricted in individuals with low total serum IgE levels.

Relationship between the IgE-specific activity level and the rate of effector cell loss with Omalizumab® treatment

While previous studies have reported the tendency for patients with high specific to total IgE ratios to have less than ideal outcomes in terms of resolving their symptoms with anti-IgE therapy [15, 16], they did not examine the impact of the IgE-specific activity level on the “rate” of effector cell function loss. We thus performed a clinical study to investigate the hypothesis that the rate of effector cell (basophil) response reduction with anti-IgE treatment slows as the specific activity of the IgE antibody response increases.

Clinical study design

Adults (aged 18 to 50 years) with a history of respiratory cat allergy were recruited with informed consent. While 18 subjects met the inclusion criteria for enrollment, the data from only 16 who completed the study are presented in Figs. 4 and 5. Design and demographics of the study subjects are described in detail elsewhere [17]. In brief, enrollment required a clinical history of seasonal or perennial allergic rhinitis for at least 2 years with or without mild persistent asthma, serum total IgE between 30 and 700 kU/L, positive cat allergen-specific IgE by ImmunoCAP >0.35 kUa/L, positive puncture skin test 3 mm wheal greater than the saline control using alum precipitated cat hair extract (Holister Stier, Spokane WA), positive basophil histamine release to cat allergen >20% of total leukocyte content or (10–19% of total content and >50% of optimal anti-IgE induced basophil histamine release) and a positive nasal allergen challenge to cat

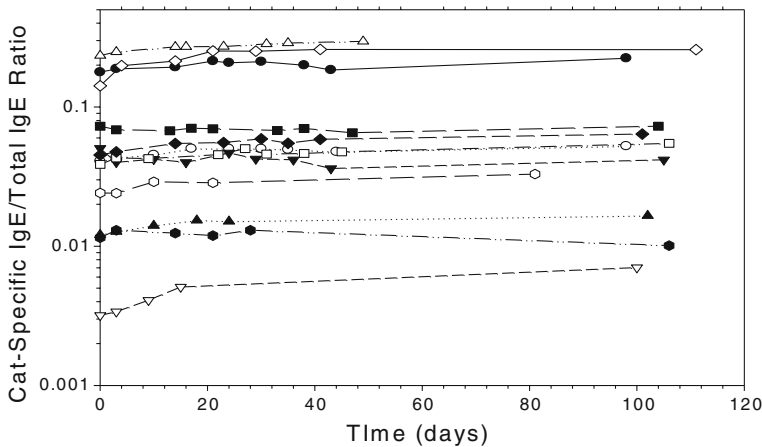


Fig. 4 Changes in serologic IgE-specific activity of 16 cat-allergic subjects receiving biweekly or monthly injections of Omalizumab[®] over a period of 16 weeks. While the total serum IgE increased in each subject by as much as 8-fold over the course of the study, the cat-specific IgE level rose in parallel with the total IgE level such that the cat-specific to total IgE ratio remained remarkably constant within an individual receiving Omalizumab[®]

allergen as defined by >5 sneezes. The 18 subjects were randomized to receive Omalizumab[®] following the manufacturer's recommended doses or placebo in a 3.5:1 ratio. Blood was collected for basophil histamine release and IgE serology at baseline and days 3, 12, 19 and 104 post initial dose. When a subject's cat allergen-induced basophil histamine release decreased to <20% of the baseline level or if not, then on study day 45, a second nasal challenge was performed to examine the clinical relevance of the basophil decrease [17].

Changes in the IgE-specific activity with increasing doses of anti-IgE

The circulating level of total serum IgE is known to increase 4–8-fold as a patient receives increasing doses of Omalizumab[®]. Our hypothesis was that the level of cat-specific IgE should increase at the same rate as the total serum IgE and thus the IgE-specific activity would be expected to remain constant over the course of Omalizumab administration, despite a 4–8-fold increase in total serum IgE. Figure 4 displays the cat-specific IgE to total serum IgE ratios in the 12 of the individuals in the clinical study who received Omalizumab[®] based on manufacturer's recommended dosing schedules every 2 or 4 weeks for a 16-week period. Within an individual who has received anti-IgE therapy, the free (Omalizumab unbound) allergen-specific to free total IgE ratio remained remarkably constant following repetitive doses of Omalizumab.

Association between IgE-specific activity level and the rate of effector cell loss following Omalizumab[®] treatment

With anti-IgE administration, there is a reduction in circulating levels of IgE that can bind to the FcεR1 on effector cells (mast cells, basophils) which leads to a reduction in cell

surface FcεR1 number and ultimately to a loss of effector cell mediator release and expression of surface activation markers. Figure 5 presents the mean (± 1 SD) cat-specific IgE in kUa/L on the ordinate versus the mean (± 1 SD) total serum IgE in kU/L on the abscissa for 16 of the 18 study subjects who completed the double-blind, placebo-controlled trial of Omalizumab[®]. Two active treatment subjects withdrew prematurely and thus basophil data were not obtained on them. The dashed lines in Fig. 5 demarcate the areas of the plot that represent 1 and 4% cat-specific IgE to total IgE ratios. The four placebo subjects (DMC, JRL, KAT, and CBD) showed no detectable decrease in their basophil histamine release over the course of the study. Four subjects receiving Omalizumab[®] (KKK, KRA, DTS and JGA), who had cat-specific IgE to total IgE ratios below 2.5%, displayed a rapid $>80\%$ decline in their basophil histamine release within 21 days of Omalizumab[®] treatment (indicated in figure with an “F” for fast). In contrast, eight subjects receiving Omalizumab[®] (SMF, TTC, RDB, MTM, TMA, JCE, SCA, and KAM) all had cat IgE-specific activities $>4\%$ and their basophil histamine release declined slowly (indicated in figure with an “S” for slow) over the duration of up to 104 days. These data support the conclusion that a high cat-specific IgE to total IgE ratio $>4\%$ is a contributing factor to the lack of effective removal of cat-specific IgE antibody from the basophil surface and the resultant lack of improvement (decrease) in the observed histamine release from the patient’s basophils following incubation with cat allergen.

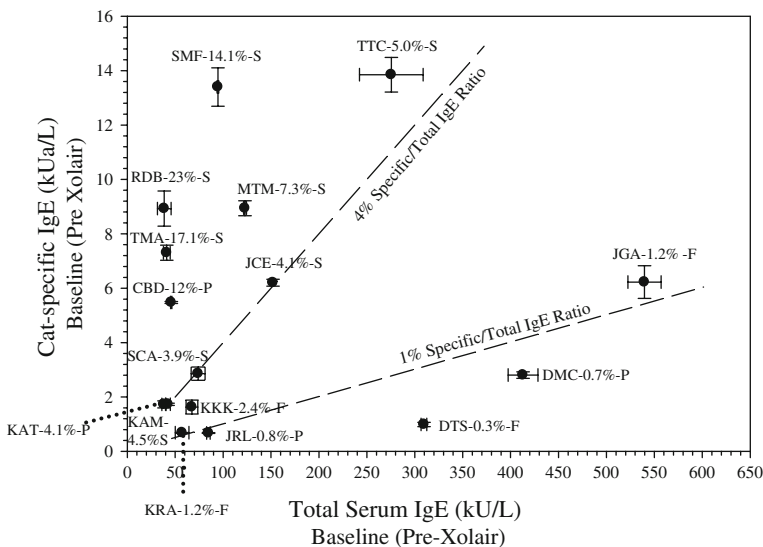


Fig. 5 Baseline (pre-Omalizumab[®]) mean (± 1 SD) cat-specific IgE levels in kUa/L versus the mean (± 1 SD) total serum IgE in kU/L for the 16 subjects who completed the study. The dashed lines demarcate the 1 and 4% specific IgE to total IgE ratios in the figure. Four placebo subjects (DMC, JRL, KAT and CBD; “P” = placebo) showed no decrease in their basophil histamine release over the course of the study. Four subjects receiving Omalizumab[®] with baseline cat-specific IgE to total IgE ratios below 2.5% displayed a rapid decline in their basophil histamine release within 21 days of starting Omalizumab[®] treatment (“F” = fast). In contrast, eight subjects receiving Omalizumab[®] with cat IgE-specific activities $>4\%$ declined the extent of their basophil histamine release more slowly (“S” = slow) over the period of up to 16 weeks

Summary and conclusion

There are multiple patient and environmental factors that influence a sensitized (IgE antibody positive) patient's predisposition to manifest allergic symptoms following allergen exposure. Among these are the magnitude and quality of the patient's humoral immune response to the offending allergens in question. The concentration, affinity and clonality of the IgE antibody response all influence how effectively the effector cells are armed with relevant IgE antibody and how well the IgE binds to allergenic molecules following inhalation, ingestion or injection. The relative level of the specific IgE to the circulating total IgE has been termed "specific activity" after a nuclear physics definition of specific radioactivity. The effect of specific activity is the least well-studied humoral immune response parameter that influences effector cell function [18]. There are a number of likely reasons for this variable having an influence on the clinical response. The simplest explanation is that a lower percentage of allergen-specific IgE on the cell surface means a lower density for making aggregates that stimulate the cell. For a given total IgE concentration, this statement will be true. However, the IgE FcεR1 receptor number on basophils responds to higher serum IgE with up-regulation of expression. This is a passive result of IgE binding that stabilizes newly synthesized unoccupied receptor on the cell surface. As the body puts out more IgE in circulation, the cells respond by putting out more epsilon chain binding receptors. If receptor expression was linearly responsive to serum IgE, then the percentage of the total IgE that is specific would not be relevant, since the same absolute density could always be obtained by up-regulation. But up-regulation is log(e)-linear, i.e., a 10-fold increase in IgE leads to only about a 2.7-fold increase in receptor expression. This means that receptor expression does not keep up with changes in total serum IgE levels and therefore irrelevant IgE can dilute out or swamp the surface receptors before specific IgE antibody can bind. This reduces its cell surface density cat-specific IgE which leads to a reduction in the cell's ability to become activated following exposure to specific allergen. Whether the clinical importance of the 4% specific IgE to total IgE ratio threshold withstands the test of future studies or not, the IgE immune response-specific activity now must be considered prominently among the many variables that influence the efficacy of management strategies for human allergic disease.

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