Accuracy of IgE antibody laboratory results

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Background: Studies have demonstrated that the magnitude of sensitization as evidenced by specific IgE (sIgE) levels provides significant information as to whether a sensitized individual is likely to be truly reactive. However, it is not clear that quantitative sIgE results provided by different laboratories using different technologies are comparable.

Objective: To investigate whether similar results were obtained from Clinical Laboratory Improvement Act–certified laboratories that used 3 common systems for sIgE antibody determination with serum samples and mouse-human IgE chimeric antibodies with known specificity and quantity.

Methods: Sixty samples for peanut and 20 for soy were submitted for sIgE determination on 3 different systems: ImmunoCAP, Immulite, and Turbo radioallergosorbent test (RAST). Mouse-human chimeric IgE antibodies specific for the major birch allergen Bet v 1 and for the dust mite allergen Der p 2 were also included.

Results: A qualitative evaluation using a cutoff of 0.35 kUA/L showed some differences in the ability to detect sIgE sensitization, with the Turbo RAST being most variable. However, considerable differences were found with quantitative evaluation, with Immulite overestimating and Turbo RAST underestimating sIgE compared with ImmunoCAP. Similar discrepancies were seen with the mouse-human chimeric IgE antibody samples.

Conclusion: These findings have potentially serious clinical implications, since each of these systems is widely used. It is therefore important that all laboratories clarify which system they are using. Just because 2 systems present their results in the same units does not mean that the results are necessarily correct or interchangeable.

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INTRODUCTION

When patients seek medical help for suspected allergic disease, possible offending allergens and the degree of sensitization must be identified. Such evaluation is usually done with either skin testing or serologic tests for specific IgE (sIgE).[1,2] Most studies to date have investigated the relationship between allergic disease and sensitization from a dichotomous (positive or negative) perspective based on arbitrarily chosen analytical cutoff points.[1–4] However, recent studies, particularly in food allergy, have demonstrated that the magnitude of sensitization as evidenced by the quantitative level of food-specific antibodies provides significant guidance as to whether a sensitized individual is likely to be truly reactive to a specific food.[5–14] Similar studies on asthma and inhalant allergy have also demonstrated the relevance of quantitative sIgE levels in the context of clinical disease.[15–19]

During the past 3 decades a variety of laboratory methods have been developed for the measurement of sIgE antibodies. Unfortunately, some studies have noted large discrepancies among results obtained by different methods.[20–24] One of the challenges in caring for patients is that physicians frequently are not able to choose their preferred method of testing because different insurance systems and health care professionals contract with different laboratories. Further confusion in this area has arisen because different manufacturers report their sIgE results in seemingly identical classes or units, which can camouflage possible differences in their performance. This problem has not been seriously addressed in detail or placed in evidence in a formalized manner, although undoubtedly this field would benefit from improved standardization.[25,26]

In this study we sought to investigate this issue by performing a blinded comparison of the results from representative laboratory systems for 2 important allergens: peanut and soy. In addition, we sought to provide insight into the reasons for discrepant laboratory values by analyzing the results from repetitive analysis of dilutions of mouse-human chimeric IgE antibodies directed against birch pollen (Bet v 1) and house dust mite (Der p 2), with defined antibody specificity and protein concentration. In such experiments, if measured correctly, the sIgE in all samples should be equivalent to the total IgE (tIgE).

MATERIALS AND METHODS

Serum Samples

Sixty routine serum samples previously tested for sIgE to peanut and 20 samples previously tested for sIgE to soy were chosen from random clinical samples sent for routine analysis in the serum bank of a commercial reference laboratory to cover the range of sIgE levels from lower than 0.1 kU_A/L up to 100 kU_A/L. All patient identifications were removed. The

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samples were thawed, aliquoted in sample tubes, and shipped according to the clinical routine for shipment of clinical samples for laboratory analysis.

Chimeric Antibodies

Mouse-human chimeric IgE antibodies specific for the major birch pollen allergen Bet v 1 and the house dust mite allergen Der p 2 were included in the study. The chimeric anti–Bet v 1 antibody was provided by Phadia AB (Uppsala, Sweden) and the chimeric anti–Der p 2 antibody originally produced by Dr Martin Chapman was purchased from Indoor Biotechnologies Inc (Charlottesville, Virginia). The scientific generation of these chimeric IgE monoclonal antibodies has been described in detail elsewhere.[27–29] In both cases the constant part of the immunoglobulin heavy chain of the original mouse monoclonal antibody has been exchanged with the constant part of the human IgE heavy chain by use of molecular biology engineering. The protein concentration of the anti–Bet v 1 construct was, according to Phadia AB, determined by amino acid analysis to be 608.25 μg/mL. The anti–Der p 2 IgE chimeric protein concentration of 10.5 μg/mL was given by the manufacturer. The translation factor among tIgE, sIgE, and protein concentration has previously been established as 1 kU IgE = 1 kU_A = 2.42 μg using ImmunoCAP.[30] Both sets of chimeric antibodies were aliquoted in human plasma that contained less than 0.2 kU/L of tIgE. Concentrations of 96.8 μg/L, equal to 40 kU_A/L, and thereafter 2-, 10-, and 50-fold dilutions were prepared. If measured correctly the obtained values would be similar for tIgE and sIgE in all these samples.

IgE Analysis

The serum samples with peanut and soy sIgE antibodies were coded and sent to 2 Clinical Laboratory Improvement Act–licensed national reference laboratories for determination of allergen sIgE by the following methods: (1) ImmunoCAP 1000 (Phadia AB), (2) Immulite 2000 Standard (Diagnostic Products Corporation, Los Angeles, California) both run in the same commercial laboratory, and (3) Turbo radioallergosorbent test (RAST) (Hycor Biomedical Inc, Garden Grove, California) run in another commercial laboratory. The samples were tested in monoplicate. The samples that contained the chimeric antibodies were randomized, coded, and sent on 3 separate occasions to the same laboratories for blinded analysis of tIgE, birch, and mite sIgE. On the ImmunoCAP and Immulite systems, duplicate samples were also analyzed for further precision analysis. The laboratory that used the Turbo RAST for sIgE used ADVIA Centaur (Bayer Diagnostics, Tarrytown, New York) for tIgE determinations. The reagents for these tests were proven by the manufacturers to be IgE specific.

Statistical Analysis

The obtained results from Immulite, Turbo RAST, and ImmunoCAP were compared. Based on its wide use and standardization both from a technical and clinical perspective as verified in more than 3,000 peer-reviewed publications, ImmunoCAP was used as the reference system. Weighted linear regressions, using weights proportional to the scales, were used for comparing the 3 systems for the peanut and soy samples. For the regression slope, 95% confidence intervals (CIs) were estimated. Bland-Altman logarithmic differences between Immulite and Turbo RAST vs ImmunoCAP were also calculated for the peanut and soy samples. Means and SDs were estimated from the logarithmic differences and compared with the expected means and SDs under the null hypothesis that no differences exist among the methods examined. The SD for the logarithmic differences would, in this case, be approximately 10% and yield control limits for the average logarithmic difference of approximately $\pm$0.03 for the peanut samples and approximately $\pm$0.05 for the soy samples.

For the chimeric samples, the average concentration for each dilution was calculated from the duplicate run performed 3 different times. For ImmunoCAP and Immulite, the within- and between-assay run coefficient of variance (CV) was estimated using restricted maximum likelihood. For Turbo RAST, the ordinary sample variance was estimated. All CVs are expressed as percentages.

RESULTS

Dichotomous Comparison of Assay Performances With Peanut and Soy Antibodies in Serum

As indicated in Table 1 using a dichotomous analysis and the well-established cutoff level of 0.35 kU_A/L of sIgE, 50 of the peanut samples were classified as positive and 10 were classified as negative by ImmunoCAP. Of these 50 positive samples, Immulite revealed 47 as positive, whereas Turbo RAST detected 39 as positive. Immulite classified all of the 10 negative peanut samples as negative, whereas Turbo RAST classified 1 of these samples as positive. Using 0.10 kU_A/L as a cutoff level, ImmunoCAP classified 55 samples as positive and 5 as negative. Immulite classified 1 of the 55 positive samples as negative and 1 of the 5 negative samples as positive. Using this cutoff, Turbo RAST classified 48 of the positive samples as positive and all 5 negative samples as negative (Table 1).

Table 1. Dichotomous Comparison of Assay Performances With Peanut and Soy Antibodies in Serum

Assays	Peanut antibodies		Soy antibodies	
	Positive	Negative	Positive	Negative
Cutoff of 0.35 kU/L				
ImmunoCAP	50	10	17	3
Immulite	47	10	16	3
Turbo RAST	39	9	12	3
Cutoff of 0.10 kU/L				
ImmunoCAP	55	5	17	3
Immulite	54	4	17	3
Turbo RAST	48	5	17	3

Abbreviation: RAST, radioallergosorbent test.