

Comparison of Immunoglobulin E Measurements on IMMULITE and ImmunoCAP in Samples Consisting of Allergen-Specific Mouse-Human Chimeric Monoclonal Antibodies towards Allergen Extracts and Four Recombinant Allergens

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Key Words

Antigen-specific immunoglobulin E · Immunoassay · Chimeric antibodies

Abstract

Background: Specific immunoglobulin E (IgE) antibody in vitro tests are performed on enzyme immunoassay systems. Poor agreement among systems has been reported and comparisons have been made exclusively with allergen extracts – not with recombinant allergens. Here we compare the ImmunoCAP and the IMMULITE systems. **Methods:** Ten patient samples with positive IgE toward egg white, birch pollen or cat or dog dander were compared using allergen extracts or the recombinant allergens Gal d 1, Bet v 1, Fel d 1 and Can f 1 with the two assay systems. Comparisons were also performed using four monoclonal mouse-human chimeric IgE antibodies specific for the same allergenic components. **Results:** IMMULITE estimated a higher allergen-specific IgE concentration in sera than ImmunoCAP when testing with allergen extracts as well as recombinant allergens. The chimeric antibodies gave an equivalent response in the total IgE and specific IgE (sIgE) with an average ratio of 1.08 (range 0.9–1.3) on ImmunoCAP. In contrast, IMMULITE ex-

hibited sIgE signals that were substantially higher than the summed level of IgE for all four chimeric antibodies (average ratio 2.96 and range 1.7–4.3). **Conclusion:** Comparison using chimeric antibodies allowed the evaluation of the true performance of the systems. ImmunoCAP measured total IgE and sIgE equally, whereas IMMULITE displayed higher sIgE signals when compared to the summed level of total IgE for all four chimeric antibodies. Results obtained with the two assay systems are not interchangeable by means of mathematical conversion.

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Introduction

Since the development of the RAST test (radioallergosorbent test) by Pharmacia, the in vitro measurement of allergen-specific immunoglobulin E levels has been a cornerstone of allergy diagnosis. The techniques have changed, and for the most important allergens, either proteins from natural sources or recombinant proteins are included in modern specific IgE (sIgE) immunoassays [1]. The predominant platforms today are IMMULITE from Siemens (formerly DPC) and ImmunoCAP from

Thermo Fisher (formerly Phadia/Pharmacia). Both allow the analysis of a variety of allergens. Studies comparing the two systems have shown relatively poor agreement [2, 3], even though it has been suggested that the results do correlate and could be converted using a simple conversion factor [4]. These studies have been performed exclusively with allergen extracts; there are no reports on a comparison using individual recombinant allergens.

Material and Methods

Patient Samples

Ten samples from anonymous patients with sIgE antibodies and symptoms of asthma, rhinoconjunctivitis and urticaria were obtained from a serum collection (Phadia AB, Uppsala, Sweden). The samples were selected for positive IgE specificity toward egg white, birch pollen, cat epithelium or dog dander and for covering the measure range for the two test systems. A negative patient sample for each of the allergens was also included in the study.

Chimeric Antibodies

Four allergen-specific mouse-human chimeric IgE monoclonal antibodies, with the constant part of the mouse heavy chain exchanged for a human counterpart, were kindly supplied by Thermo Fisher (Uppsala, Sweden). The antibodies reacted specific with the Gal d 1, Bet v 1, Fel d 1 and Can f 1 epitopes of egg, birch, cat and dog dander and were used as reference antibodies.

IgE Analysis

Undiluted single measurements of total IgE and sIgE against the allergen extracts egg white f 1, birch pollen t 3, cat dander e 1 and dog dander e 5 and the recombinant allergens Gal d 1, Bet v 1, Fel d 1 and Can f 1 were determined by means of the IMMULITE 2500 (Siemens, Tarrytown, N.Y., USA) and the ImmunoCAP 250 (Phadia AB) systems (table 1). Both assay systems are calibrated to the WHO 75/502 IgE standard, and report results in kU_A/l [5]. The assays were performed at the Gentofte Hospital (IMMULITE) and at the Thermo Fisher scientific laboratory (ImmunoCAP) in Copenhagen, Denmark. All assays were performed in accordance with the manufacturer's instructions and the principles of good laboratory practice.

Statistical Analysis

SAS version 9.2 (SAS, Cary, N.C., USA) was used for statistical analyses using ImmunoCAP results as a reference. A difference plot (Altman-Bland) was constructed to identify between-method differences.

Results

IMMULITE gave, on average, 62% higher values than ImmunoCAP for the allergen extracts (egg white f 1, birch pollen t 3, cat dander e 1 and dog dander e 5) with patient

Table 1. Allergen-specific immunoglobulin E analysis

Patient samples (n = 10)		Chimeric antibodies (n = 4)
Allergens extracts	Recombinant allergens	Recombinant allergens
Egg f 1	Gal d 1 (ovomucoid)	Gal d 1 (ovomucoid)
Birch t 3	Bet v 1	Bet v 1
Cat e 1	Fel d 1	Fel d 1
Dog e 5	Can f 1	Can f 1

sera, (fig. 1, closed symbols). This difference was most pronounced at high values. An even more noticeable difference was observed with recombinant allergens (Gal d 1, Bet v 1, Fel d 1 and Can f 1) (fig. 1, open symbols). At values >5.0 kU_A/liter, nearly all of the observations were more than twice as high on IMMULITE as on ImmunoCAP. Closer to the cut-off limit, IMMULITE still gave higher values, but to a lesser degree on the recombinant allergens, except for Bet v 1. On the contrary, for the allergen extracts in the lower measuring range, IMMULITE gave lower results than ImmunoCAP more often. Across the four recombinant allergens, a geometric mean ratio between IMMULITE and ImmunoCAP of 1.75 with a 95% prediction interval of 0.12–24.9 could be observed.

The chimeric antibodies yield an approximately equivalent response on ImmunoCAP in both total IgE and sIgE with an average ratio of 1.08 (range 0.9–1.3) (fig. 2, inset). In contrast, IMMULITE gave sIgE signals that were a substantially higher titer of the summed IgE for all four chimeric antibodies (average ratio 2.96; range 1.7–4.3) (fig. 2).

Discussion

In spite of the relatively small number of patient samples included in this study, we were able to confirm the previous observed difference revealed by former studies using allergen extracts comparing the two immune assay systems for all four allergen extracts, especially with samples at sIgE levels >5 kU_A/liter. IMMULITE has been shown to detect fewer positive reactions than ImmunoCAP toward cereals and peanuts and was unable to detect any positive reactions toward oats [6]. It has been suggested A mathematical conversion of the IMMULITE and ImmunoCAP values has been suggested, based on observations for four food allergens extracts [4]. This approach has later been criticized [7], mainly due to the large individual variability. For example, an

Fig. 1. Difference plot between ImmunoCAP and IMMULITE/ImmunoCAP ratios, based on the sIgE analysis toward Can f 1, Fel d 1, Gal d 1 and Bet v 1 recombinant allergens (open symbols) and toward dog, cat, egg and birch allergen extracts (closed symbols). The number of observations with geometric means for the IMMULITE/ImmunoCAP ratios together with the 95% prediction interval [an estimate of an interval in which future observations will fall, with a certain probability ($p = 0.95$)] for each studied allergen appears at the top of the figure.

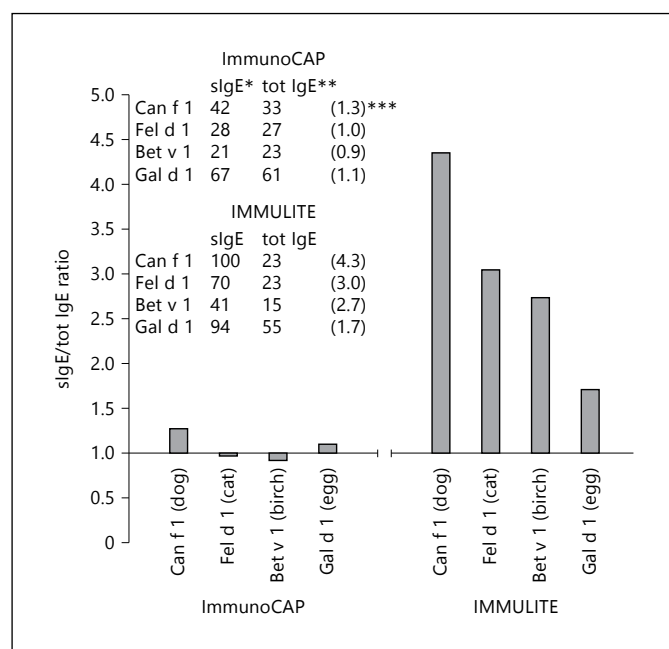
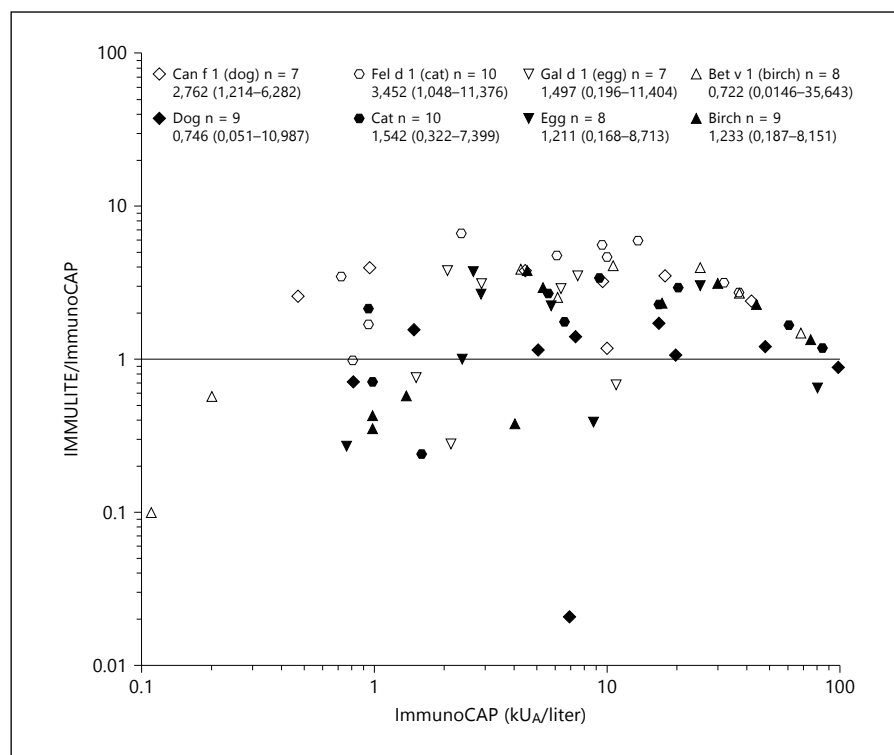


Fig. 2. sIgE/total IgE ratio toward Can f 1, Fel d 1, Gal d 1 and Bet v 1 recombinant allergens for four monoclonal mouse-human chimeric IgE antibodies. The closer to the value of 1 at the Y-axis the more accurate is the test. The observed levels of sIgE and total IgE for each of the chimera appear at the top of the figure. * sIgE = specific IgE; ** tot IgE = total IgE; *** ratio specific IgE/total IgE.

intersystem factor of 4.85 was suggested for IgE to egg white, regardless of the level of IgE. In this study, we could not verify this approach. We determined a geometric mean ratio of 1.21 with a 95% prediction interval of 0.17–8.71 (fig. 1), indicating a rather poor relationship between the two systems. The difference in response levels between the two assay systems for patient samples is more pronounced when measuring with recombinant allergens (Gal d 1, Bet v 1, Fel d 1 and Can f 1) rather than allergen extracts. Thus, the great interval demonstrates that results from one system will not be predictive of results from the other system.

An allergen-specific mouse-human chimeric IgE antibody is by definition monoclonal, i.e. with a single specificity, but is still detected by the human IgE chain part of the antibody. This allows for preparation of artificial samples where the levels of both total IgE and sIgE can be predetermined because the results should be equal or close. Comparing the results for component-specific IgE allergen with those for specific mouse-human chimeric IgE antibodies allowed us to evaluate the true performance of the two systems. However, despite covering the entire measuring range, the relatively small number of samples and the single-run analysis limited statistical evaluations. Nevertheless, the observations are

consistent with other studies reporting higher sIgE levels in the high range and lower levels in the low range by IMMULITE [7]. Furthermore, the purpose of the study was to compare the quantitative performance between the assays. To which degree these difference influence the clinical evaluation of patients requires more study material.

Clinical decision points based on sIgE with a high 95% positive predictive value have been suggested for allergies to several foods [8, 9]. In children with suspected egg allergy, it has been suggested that measurements of sIgE to ovomucoid with levels >11 kU_A/liter can be indicative for egg allergy [10]. In that study, sIgE levels were measured with ImmunoCAP, and the identified decision points could guide physicians in performing food challenges.

As the chimeric IgE antibodies are presumably selected for reactivity toward the recombinant allergens produced by Phadia/Thermo Fisher, different results may have been obtained if they were selected for reactivity toward Siemens allergens. However, the observed differ-

ences clearly reveal that the two assay systems are not interchangeable, probably caused by differences in allergens, conjugation chemistry and lack of standardization. Furthermore, a derived decision point determined on ImmunoCAP cannot be applied to results obtained by IMMULITE.

In summary, our findings indicate that the results of the two different systems investigated cannot be used interchangeably; the levels of serum IgE and the predictive value associated with the clinical evidence of allergy obtained with one system cannot be compared with results for the other system. This has also been noted in the guidelines of the National Institute of Allergy and Infectious Diseases.

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