

ORIGINAL ARTICLE

Immunological measures as potential markers of dose

J. Kleine-Tebbe

Allergy & Asthma Center Westend, Berlin, Germany

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Correspondence

Jörg Kleine-Tebbe, Allergy & Asthma Center Westend, Berlin, Germany.
E-mail: kleine-tebbe@allergie-experten.de

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Abstract

Specific immunotherapy (SIT) induces immunological changes leading to a decrease in allergen-driven symptoms and medication use. However, the use of arbitrary and variable assay formats and units hampers comparisons between SIT studies. The magnitude of IgG response induced by SIT is not directly linked to clinical improvement, so an increase in allergen-specific IgG₄ should be considered a necessary but not sufficient marker of clinical efficacy. Dose-dependent IgG₄ immune responses should be demonstrated by the manufacturer, not by doctors. Symptom and medication scores are essential end-points for immunotherapy studies, but are subjective, so close association with objective assay measurements will be difficult. Nonetheless, blocking antibody assays combining serology and cellular function may provide a solution for relating immunological assay measurements to clinical outcomes.

IgE-mediated reactions have shaped our understanding of allergen-driven dose-responses

Dose–response studies conducted in our laboratory over 30 years ago confirmed that allergen-induced IgE-related responses – illustrated by basophil histamine release curves – are variable, heterogeneous and complex. These features have shaped our understanding of dose–response relationships in immediate-type hypersensitivity induced by protein allergens. Although these assays are reproducible, give large plateaus and are analytically sensitive to picograms of allergen, they reflect only sensitisation and do not necessarily correlate with clinical parameters. After 25 years, the conditions that explain this variability are being dissected. Dose–response analysis (1) showed that basophil degranulation assays are affected by factors such as a) the total IgE loaded onto the cells; b) the fraction of specific IgE relative to total IgE; c) antibody avidity; and d) clonality (ability to bind to one or many epitopes). Using Der p 2 as a model allergen and monoclonal antibodies to Der p 2, Christensen *et al.* applied Biacore technology to measure the antibody affinity of different clones, and the results showed that covering the antigen with more antibodies gives a stronger response. These tools have therefore been of great help in dissecting complex human cellular responses. In contrast to the broad variability seen in effector cell responses during immediate-type allergic reactions, a rather narrow range of 5–20 µg of major allergen is believed to have an effect (2) when applying subcutaneous immunotherapy (SCIT) (3).

Markers of a defensive versus tolerogenic immune response

During IgE-mediated defence, for example against parasites, neutrophils, eosinophils, basophils and lymphocytes are the primary protectors of the immune system. As for all immune responses, their action needs to be controlled. In allergy, they cause damage and destruction of airway tissue through inflammation with subsequent nonspecific mucosal hyperreactivity, long from the goals of successful SIT. Therefore, to monitor SIT, we need an immunological marker of tolerance and response suppression that is robust and reliable, feasible and reproducible, quantitative, allergen specific and clinically relevant. However, cell-based assays using dendritic cells or T-cell-based assay systems are not yet appropriate for quantitative measurement of responses. They are too variable, lack validation and standards and are not yet associated with clinical outcomes.

Because T cells are sometimes in a resting state and sometimes activated, looking at serological markers may be more useful. Long before IgE was discovered, blocking antibodies against ragweed pollen, important allergen sources in the US, were studied by Robert Cooke and Mary Hewitt Loveless. A high-dose group treated with ragweed extract showed an IgG response against the purified major ragweed allergen, in the first example of an antigen-specific response to SIT. This has also been achieved with purified allergen, allergoids, and recombinant allergens (4–7) and with allergens from cat, dog and house dust mites. However, individual heterogeneity in

the immune response is observed, and IgG responses are not always strictly dose related (8–10). The pattern is similar with sublingual immunotherapy (SLIT), which also demonstrates an IgG response (11–12). This shows that similar effects can be obtained from different application routes, because SLIT is no systemic treatment like SCIT, but still gives a dose-dependent IgG effect. These studies used relative units so comparisons between studies are difficult and standardisation is needed.

Standardisation of allergen-specific IgG assays and results

One solution is an assay that uses solid-phase anti-IgG₄ to capture all IgG₄, using a standard reference either from myelomas or an international reference serum. Quantification of captured antibodies is obtained with a second anti-IgG₄ labelled for detection. This is similar to a common laboratory assay for total IgG antibody and can be used to generate a standard curve. For specific IgG₄ measurements, allergen is used as the solid phase, and bound IgG₄ is measured in mass units using the standard curve. However, this 'heterologous calibration' method requires that the solid-phase capture anti-IgG₄ and the solid-phase allergen have the same affinity for IgG₄. Another approach uses solid-phase anti-IgG₄ as a capture antibody for measurements of standards or serum samples. In this case, the standard is a chimeric antibody whose specificity is dictated by the mouse monoclonal component. The advantage is that the standard can be used at the same concentration in assays for total IgG₄ or specific IgG₄. Specific IgG₄ is then detected by labelled allergen.

This assay was used to show individual responses of IgG₄ to Group 5, the major grass allergen (5). In some patients, Group 5-specific IgG₄ covers almost the entire IgG₄ response, indicating IgG production primarily to the major, immunodominant allergen family. However, IgG₄ becomes monovalent *in vivo* (13), and understanding how this recombination process translates into clinical effect for patients is difficult. Also, depending on the study and preparation used, IgG₄ responses can be highly variable, with very high responses for recombinant SCIT allergens. We do not know how high a response must be to indicate or initiate clinical effects. Therefore, clinical outcomes are not yet convincingly related to increases in IgG₄. This parameter is probably necessary for clinical efficacy; however, it is not necessarily sufficient (see Table 1). In contrast, an absence of a rise in IgG₄ would raise questions about the utility of a SCIT product, but manufacturers rather than doctors should ensure that products produce a rise in IgG₄.

Blocking-IgG antibody and functional assays with cellular readouts

Blocking antibody assays may test for both presence of antibodies and function. Three types of blocking or functional tests involving cellular components exist. One measures the ratio of IgE binding to allergen with or without the presence of the competing IgG and is similar to a total IgE test with

Table 1 Features and conclusions regarding IgG₄ assays for monitoring subcutaneous immunotherapy (SCIT)

Positive features of IgG ₄ assays	Difficulties of IgG ₄ assays and interpretation
Robust and feasible	Different assay formats
Reproducible	Not associated with clinical outcome?
Quantitative	Missing general standardisation
Allergen specific	Only meaningful if determined towards immunodominant major allergens
Dose dependent	Clinically relevant?
Rise in IgG ₄ : necessary, although not sufficient for successful immunotherapy	No rise in IgG ₄ : raises questions about the utility/dose of the SCIT product
Conclusion: The allergen manufacturer, not the doctor, should check for IgG ₄ rise after application of SCIT with a defined dose	

or without the washing step. The two others are facilitated-antigen presentation (FAP), and the basophil activation test (BAT). A study on SCIT showed a change in IgE-blocking antibodies by all three tests (14). FAP relies on the low-affinity IgE receptor CD23 on B cells. The assay allows B-T-cell contact and measures T-cell activation (15, 16). In IgE-facilitated allergen binding (FAB), the readout is binding of allergen-IgE complexes to B cells after binding or blocking, measured by flow cytometry (17, 18). A study with two patient groups receiving two different doses of SCIT showed a dose-related increase over time in IgG₄, both by functional and binding assays. This also paralleled a rise of nearly a factor of 10 in IgE (19). A weak but highly significant association was seen with clinical results determined by symptom and medication scores after SCIT. This shows that the FAB assay and IgE-blocking factor are more closely related to clinical outcomes than IgG₄, which was measured in parallel. Combining serology and cellular assays appears to be a superior solution for solving the problems of relating assays to clinical outcomes. Nonetheless, symptoms and medication scores are soft measures that are variable, heterogeneous and subjective, so the relationship between symptoms and recorded medication scores is not yet clear. High-tech tools were used to show that for FAP, varying the avidity of an antibody and the number of binding sites for allergen varied the global response (20). Using different ratios of IgE/IgG or diluting the IgE also altered the response.

In conclusion, standardised cellular assays are difficult to achieve and currently too variable to determine effectiveness or dose-dependent effects of SIT. IgG₄ to major allergens will rise after SCIT and SLIT, and although the mere quantitative increase does not reflect responses to SIT, the functional effects of SIT-induced IgG responses show promise as a useful marker of SIT. Dose-dependent IgG₄ immune responses

should be demonstrated by the manufacturer, not by doctors. Functional assays that use both cell and serological components might best reflect the clinical situation. According to the authorities (European Medicines Agency) and our current concepts, immunotherapy studies should be based on symptom and medication scores. However, these represent in part subjective parameters, so close association with objective immunological measures will be difficult to achieve.

Conflict of interest

Jörg Kleine-Tebbe has received research grants from Allergopharma, ALK-Abelló, Dr. Focke, HAL Allergy and Phadia, consultancy fees from ALK-Abelló, Bencard, HAL Allergy, Novartis and the Paul-Ehrlich-Institut as well as lecture fees from Allergopharma, ALK-Abelló, Bencard, HAL Allergy, Leti, Novartis, Phadia, Roxall and Stallergenes.

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