

IgE antibody concentration, specific activity, clonality, and affinity measures from future diagnostic confirmatory tests

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For years, allergy researchers have been investigating the constellation of parameters that modulate effector cell mediator release. Their goal has been to devise new strategies for reducing IgE-mediated responses in sensitized individuals after allergen exposure. The article by Christensen et al¹ in this issue of the *Journal* provides clarity to the humoral immune response component of the allergic response through empiric data that show that concentration, specific activity (specific to total IgE ratio), affinity, and clonality (epitope specificity) of the IgE antibody response all work together to affect effector cell activation. In this study the authors use an impressive combination of molecular biology, immunochemistry, cellular biology, and chip-based surface plasmon resonance techniques to prepare and characterize a unique panel of IgE anti-Der p 2 antibodies. Surface plasmon resonance technology allowed the authors an effective means of characterizing the interaction of Der p 2 with IgE anti-Der p 2 in real time. Surface plasmon resonance permits this by attaching one interacting partner (Der p 2) to the surface of a sensor chip and passing a sample containing the other interacting partner (IgE anti-Der p 2) over the surface. In the case of the study by Christensen et al,¹ binding of IgE anti-Der p 2 to insolubilized Der p 2 generated a measured response that is proportional to the bound mass. This analysis allowed the authors to accurately characterize the specificity, concentration, affinity, and clonality of their unique panel of Der p 2-specific IgE antibodies. The authors prepared a panel of 10 mouse/human chimeric IgE antibodies and 9 fully human Der p 2-specific recombinant IgE antibodies that had been cloned from the B cells of a patient with dust mite allergy by using a phage display technique. This unique antibody panel allowed the authors to perform a flow cytometry-based basophil activation test using combinations of IgE antibody affinities ranging from 0.0358 to 291 nmol/L with up to 9 distinct Der p 2-binding epitopes. The authors used basophils from nonatopic donors. All the antibodies of the different affinities or clonalities were studied by using a common basophil preparation to minimize potential variability associated with the basophil source

(nonatopic vs atopic donor). The authors also adjusted the Der p 2-specific IgE/total IgE ratio by adding varying amounts of non-Der p 2-specific IgE. For possibly the first time, the authors were able to simultaneously control the IgE antibody concentration, affinity, clonality, and specific activity added to the basophil activation effector cell model. With this unique flexibility, they were able to control for the actual IgE antibody characteristics and empirically show that higher levels of basophil activation occurred with (1) higher total IgE levels, (2) higher specific activity or Der p 2-specific/total IgE ratios, (3) broader clonality, and (4) a higher-affinity IgE antibody.

The future diagnostic implications of these observations are profound. Allergy diagnosis should always begin with the history and physical examination. However, once a history suggests a high probability of allergic disease, future diagnostic confirmatory tests for IgE antibody need to provide more sophisticated measures of the patient's IgE antibody profile. The confirmatory test for sensitization (IgE antibody) should provide as much information as possible about the patient's IgE antibody concentration, clonality-specificity, affinity, and specific activity. The bioassay (skin test) is powerful in its ability to integrate all these parameters to provide a biologically relevant IgE response as a visually measured wheal or erythema. However, it is unlikely that the skin test will be able to provide detailed information about the concentration, clonality, affinity, or specific activity of a particular patient's IgE antibody response.

In contrast, serology provided by a Clinical Laboratory Improvement Act of 1988—certified diagnostic allergy laboratory is uniquely poised to provide this information. Presently, the serologic assessment of IgE antibody in allergic patients has technically advanced since its introduction in 1967; however, it is still in its infancy. We measure specific IgE antibody levels to defined allergen mixtures in quantitative international units per milliliter, which is a vast improvement over the historical qualitative (positive/negative) and semiquantitative IgE antibody class levels of the past.² Occasionally, total serum IgE levels are measured, most commonly to assess less common diseases (eg, allergic bronchopulmonary aspergillosis and Job's syndrome) associated with high total serum IgE levels or to identify patients who are appropriate for treatment with omalizumab. Therefore at present information about the concentration of IgE antibody for a specific allergen is limited, and no information is provided from current serology about the patient's IgE antibody clonality, affinity, or specific activity. Even with this rather crude measure of the patient's IgE antibody response, quantitative specific IgE antibody levels have been shown to be useful in predicting the probability of clinically relevant food allergy^{3,4} and wheeze in children.⁵⁻⁷

However, the design of allergen-specific IgE antibody assays is changing rapidly, and future assays involving automated chip-based arrays of purified and recombinant allergens should be able

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to more effectively dissect the IgE antibody response of an individual patient. We know that at least 1 IgE antibody assay is quantitative such that 1 IU of allergen-specific IgE bound is equivalent to 1 IU of total IgE bound.⁸ Thus by analyzing total serum IgE and allergen-specific IgE antibody levels, the IgE antibody-specific activity (allergen-specific IgE/total IgE ratio) and concentration can be accurately measured. The biologic importance of this specific IgE/total IgE ratio was first put forth in 1993 by MacGlashan⁹ using the basophil as the model effector cell. More recently, IgE antibody-specific activity has been shown to be clinically important in the assessment of diagnostic tests using effector cells, such as basophils,¹⁰ and clinically in the assessment of asthmatic patients.¹¹ Conceptually, if all else is equal in 2 individuals except for the allergen-specific IgE/total IgE ratio, the individual with the higher specific/total IgE ratio will have a higher probability for effector cell activation when exposed to a common allergen source. The higher specific IgE/total IgE ratio increases the probability that 2 IgE molecules with the same allergen specificity will be docked in their receptor close to each other on an effector cell *in vivo* or *in vitro* and thus be more readily cross-linked by a given allergen molecule. In the future, diagnostic laboratories might elect to provide an allergen-specific IgE antibody result to clinicians both as a quantitative kilounit per liter measurement and as a specific IgE/total IgE ratio.

One can add the power of the Immuno Solid Phase Allergen Chip¹² (UBC Genomics, Vienna, Austria) technology, in which purified allergens are spotted in triplicate arrays, to the current IgE antibody serologic methods. This technology provides additional information about IgE antibody clonality by using individual native or recombinant allergens, such as the Der p 2 panel from the study by Christensen et al¹ that were genetically modified to display a different repertoire of allergenic epitopes. Moreover, it is theoretically feasible that by spotting different concentrations of group 1 allergens on the chip, relative IgE antibody affinity can also be evaluated. This technology has been applied in Europe to provide more comprehensive diagnosis and management of human allergic disease.¹³

The data of Christensen et al¹ also have implications for the therapeutic management of allergic patients. Their study further emphasizes how the systematic modification of allergens, such as Der p 2, by using molecular biologic methods, can alter allergenic epitopes in a manner to reduce effector cell activation while potentially maintaining regulatory T-cell expansion. Although this strategy might facilitate the induction of reduced sensitization with fewer adverse reactions, the effect of modified allergen use on cytokine generation (IL-3 and TNF- α) and effector cell survival¹⁴ will need to be further investigated.

A missing issue of the study by Christensen et al¹ is the role that natural exposure or allergen immunotherapy-induced specific non-IgE antibodies play in modulating the basophil response. Although there is an extensive literature on naturally occurring anti-human IgE antibodies, Lichtenstein et al¹⁵ showed that most individuals have IgG antibodies that are either directly bound to IgE or bound to allergen that is complexed with IgE

resident in the Fc ϵ RI receptor. Anti-human IgG mAbs were able to cross-link and induce basophil mediator release. Moreover, the IgG subclass specificity of this response was shown to be consistent within a given allergic patient over time. One future direction for the authors would be to prepare a comparable panel of humanized IgG chimeric and engineered antibodies with the same Der p 2 specificity used in the current study. They could then systematically show the extent to which non-IgE antibodies alter effector cell activation through either competitive inhibition or cell-surface γ receptor binding.

The authors are to be commended for their systematic work in empirically confirming the interplay of IgE antibody concentration, specificity, specific activity, and affinity in effector cell activation.

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