

EDITORIAL

Clinical & Experimental Allergy

Old questions and novel clues: Complexity of IgE repertoires

This editorial discusses the findings of the paper in this issue by N. Willumsen et al. [1], pp. 1227–1236.

J. Kleine-Tebbe

Allergy & Asthma Center Westend, Outpatient Clinic Hanf, Ackermann & Kleine-Tebbe, Berlin, Germany

The transition from IgE-mediated sensitization to clinical allergy symptoms still remains the million dollar question for practicing allergists and clinical scientists. How can the diversity of allergic (cellular or mucosal) responses and clinical symptoms be explained facing i.e. identical serum levels of allergen-specific IgE or identical skin test responses to common atopic allergens?

The article by Willumsen et al. [1] provides new clues about the relationship between allergen-specific IgE quantity and IgE repertoire complexity, composed of the number of recognized epitopes plus the affinity of the IgE involved. The study is based on powerful research tools, designed and successfully tested by Lars H. Christensen for his PhD thesis [2]: A broad panel of recombinant, human, monoclonal IgE antibodies with different affinities to various epitopes of Der p 2, a well-defined major allergen of house dust mite *Dermaphagoides pteronyssinus*.

For the study reported in this issue [1], different sera from house dust mite-sensitized individuals were tested to form IgE-Der p 2-CD23 complexes on B-cells, termed Facilitated Antigen Binding (FAB), an important IgE-mediated pathogenic mechanism in allergic diseases [3]. Adding synthetic, epitope-specific IgE of defined affinities indirectly uncovered the general sera's IgE antibody repertoire: Only sera with high Der p 2-specific IgE formed complexes without further added synthetic IgE. Moderately elevated Der p 2-specific IgE containing sera variably facilitated complex formation, depending

on the affinity and number of epitopes of the added monoclonal IgE. Sera with low Der p 2-specific IgE titres were unable to induce FAB; only two added IgE species of high affinity could dramatically enhance complex formation.

In conclusion, the authors established an indirect approach by adding epitope-specific monoclonal IgEs of defined affinities in FAB assays to explore the complexity of allergen-specific serum IgE repertoires. Elevations of allergen-specific IgE came along with enhanced epitope coverage, increasing the complexity of the IgE repertoire.

Is it necessary to use such synthetic material? Yes, absolutely! Purification of IgE from serum is feasible, but cumbersome [4] and could never provide such unique reagents, being applied by the authors. What are the potential implications? Improved insights into the composition and the complexity of individual allergen-specific IgE repertoires might establish new scientific tools for diagnostic, prediction and monitoring purposes. Allergen-specific IgE repertoires in relation to its quantity and to total IgE levels [5] might help to predict the occurrence, persistence and/or the severity of allergic symptoms and IgE-mediated hypersensitivity reactions, in other words the progression of allergic conditions and diseases.

Using defined allergen-specific recombinant IgE and IgG antibodies, the research team of Kaare Lund could already demonstrate that IgE clonality rather than affinity controls FAB complex formation, especially with medium- and low-affinity IgE antibodies and that differences in allergen-specific IgE affinity determine subsequent T-cell activation. Moreover, the complexity of an IgE repertoire did affect the ability of allergen-specific IgG antibodies to inhibit FAB [6].

This concept will influence our understanding of allergen-specific immunotherapy, being accompanied by an increase in antigen-specific IgG₄ antibodies with blocking capacities. In case of rather complex IgE rep-

Correspondence:

Dr. J. Kleine-Tebbe, Allergy & Asthma Center Westend Outpatient Clinic Hanf, Ackermann & Kleine-Tebbe Spandauer Damm 130, Haus 9 D-14050 Berlin, Germany. E-mail: kleine-tebbe@allergie-experten.de

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ertoires it might become more difficult to successfully compete, inhibiting functional interactions like FAB and subsequent T-cell responses. In contrast, in patients with a rather simple IgE repertoire with limited clonality and affinity, allergen-specific immunotherapy with subsequent IgG₄ increases might be more effective to alter the course of the disease.

Previous studies have demonstrated the influence of the IgE repertoire, its affinity and its epitope coverage, on dose-dependent responses of passively sensitized basophils using synthetic IgE panels to Der p 2 from the tool box of Lars H. Christensen [2].

The response of the basophils, used as effector cells, was effected by:

- the total amount of passively sensitized IgE (number of occupied IgE-receptors),
- the ratio of specific to non-specific IgEs (or fraction of specific to total IgE),
- the ratio of IgE to two different close or distant epitopes,
- the number of epitopes covered by the synthetic IgE (IgE repertoire),
- the abundance of IgEs with high, medium or low affinity.

This systematic work was praised [7] for good reasons, demonstrating convincingly the complex relationship of IgE antibody concentration, specificity, ratio of specific to total IgE, epitope coverage and affinity in effector cell activation. Presumably, antigen-specific IgG might add another degree of complexity leading to blocking effects and potentially inhibitory signals, which have not studied in the effector cell system by the authors yet.

In addition to these purely serologically defined variables, some cellular components have been uncovered by others to influence effector cell functions, including self-limiting basophil signals [8], adding to the broad variability of allergen-dependent IgE-mediated responses (reviewed in [9]):

- Total basophil IgE receptor cell surface density, i.e. number of FcεRI receptors/cell, being regulated by total tissue (and serum) IgE [10],
- intrinsic cellular sensitivity of the basophils, i.e. number of IgE molecules required for 50% of the maximal cellular response [11],
- cellular reactivity of the basophils (maximum cellular response after optimal IgE-mediated stimulation, i.e. with polyclonal anti-IgE), determined by signalling through tyrosine kinase Syk [12, 13].

These cellular variables add another degree of complexity to simple serological measurements and should be considered understanding mast cell-based skin test responses, too.

Immunoglobulin repertoires have also been studied in relation to allergen isoforms [14]: Individual recombinant IgE clones from a subject allergic to house dust mites recognized Der p 2 with affinities varying up to 100-fold between different Der p 2 isoforms. Applying human basophils passively sensitized with limited rIgE repertoire, cellular responses corresponded to rIgE affinities to certain Der p 2 isoallergens. In contrast, basophils sensitized with polyclonal patients' sera showed identical responses to different Der p 2 isoallergens. Obviously, certain IgE antibodies can bind single allergens with a broad range of affinities due to natural isoallergen variations. The subsequent increase in complexity of interactions between IgE and allergen at the effector cell surface might, however, become indistinct due to the nature of patients' polyclonal IgE. Subsequently, characteristics of the allergen will clearly impact on the global allergic (cellular) response:

- Biochemical and structural features of the allergen (number and proportion of single molecules including isoforms in a complex allergen mixture; number of potential IgE-binding epitopes of a single molecule and their affinities),
- nature of the aggregates formed by complexes of (single) allergens and IgE (i.e. dimers, trimers, oligomers).

Why have things to be so complicated? Because simple answers do not necessarily provide satisfactory explanations! With the aforementioned antigenic, serological and cellular variables we can sketch a more complete picture and can finally appreciate and accept the:

- missing association between quantitative allergen-specific serum IgE levels to a single molecule (i.e. demonstrated with major birch pollen allergen Bet v 1) and quantitative cellular (basophil) test results, i.e. histamine release, using the same reagent [15],
- missing association between quantitative allergen-specific serum IgE levels and quantitative skin test responses (i.e. titrated intradermal tests with Bet v 1) [15], and
- The only moderate association between cellular (basophil) test results and quantitative skin test responses (being both based on cellular effector cells: basophils vs. mast cells) [15].

To avoid any misunderstanding, our present diagnostic allergy tests even with commonly used complex allergen mixtures (extracts) generally perform sufficiently enough with fair and acceptable *qualitative* concordance (positive vs. negative). A positive skin test, indirectly reflecting functional allergen-specific IgE on cutaneous mast cells, is likely to be backed up by a

positive serum IgE result and vice versa. The same applies to negative skin tests and a lack of specific IgE in serum, as long as the total IgE is high enough, the allergen preparation contains enough of the culprit allergen and gives access to all IgE-binding epitopes.

However, nobody should expect close *quantitative* associations between these tests. Considering the underlying, partially inter- and independent variables it would certainly be a surprise to uncover close correlations between quantitative "allergy test" results, which should rather be termed "sensitization tests", as none of them is able to clearly separate "clinically silent" from "clinically relevant" sensitizations with corresponding symptoms.

There are even more clues after appreciating the complexity of polyclonal IgE repertoires and subsequent cellular responses in humans. Finally, we can understand and accept:

- The lack of association between quantitative allergen-specific IgE concentrations and clinical symptom severity to inhalant allergens,
- The lack of association between quantitative skin test responses (i.e. average hive diameter or end-point titration) and clinical symptom severity,
- The lack of association between quantitative cellular (i.e. basophil) responses and clinical symptom severity,
- The difficulties to define decision points for allergen-specific IgE levels or skin test results to certain foods for the prediction of a positive challenge test in sensitized subjects, and
- The general lack of "sensitization test" outcomes with clinically relevant allergic symptoms.

The latter ones, determined clinically by the physician with information from case history and/or challenge tests, are often separated from "silent sensitizations" by brisk dichotomy. More likely, allergic individuals will experience a continuum from being sensitized to protein allergens to developing clinical symptoms and disease during the natural course of the evolving IgE response and additional alterations of the mucosal barrier.

Having a hard time to grasp the listed details and how to cope with discordant diagnostic results in allergic individuals? Don't worry. The answer will likely be: Hey, it's complex! Did you ever check for *specific to total IgE-ratio*, *epitope repertoire* and *affinity*? If not, don't bother, since you are missing parts of the picture. Looking at quantitative IgE levels, carefully measuring skin test responses or counting basophil activation read-outs is fine, but not enough – the nature of the polyclonal IgE response with its complex repertoires goes beyond our present diagnostic routine tests.

Future science might provide new tools to properly characterize and understand the complexity of the IgE repertoire of our patients during the diagnostic work-up. That will likely open new horizons for improved diagnostics for prediction and monitoring purposes, understanding and potentially modifying the path from silent sensitizations to relevant allergic symptoms.

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