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In vitro diagnosis of IgE-mediated allergy: breakthroughs in the last decade

Expert Rev. Clin. Immunol. 8(1), 9–11 (2012)



Didier G Ebo

Author for correspondence:
University of Antwerp,
Faculty of Medicine,
Department Immunology–
Allergy–Rheumatology,
Campus Drie Eiken T4.08,
Universiteitsplein 1,
B 2610 Antwerpen, Belgium
Tel.: +32 3265 2595
Fax: +32 3265 2655
immuno@ua.ac.be



**Margo M
Hagendorens**

University of Antwerp,
Faculty of Medicine,
Department Immunology–
Allergy–Rheumatology,
Campus Drie Eiken T4.08,
Universiteitsplein 1,
B 2610 Antwerpen, Belgium



Chris H Bridts

University of Antwerp,
Faculty of Medicine,
Department Immunology–
Allergy–Rheumatology,
Campus Drie Eiken T4.08,
Universiteitsplein 1,
B 2610 Antwerpen, Belgium



Wim J Stevens

University of Antwerp,
Faculty of Medicine,
Department Immunology–
Allergy–Rheumatology,
Campus Drie Eiken T4.08,
Universiteitsplein 1,
B 2610 Antwerpen, Belgium

Background

This article briefly reviews the historical development, present status and perspectives of the diagnosis of IgE-mediated allergy. After more than a century of relying on skin testing and 40 years of relying on quantification of specific IgE antibodies for the diagnosis of IgE-mediated allergy, clinicians now have access to different novel *in vitro* diagnostics in the form of component-based assays and flow-assisted quantification of activated basophils. Although these techniques already constitute key advances in the diagnosis of allergy, it is anticipated that future developments, such as epitope mapping and identification of novel basophil activation markers, might further improve management of the allergic patient. In this context, the recent development of a flow cytometric technique to analyze histamine release at a single cell level might represent a significant step forward.

IgE-mediated allergies constitute an important and increasing health problem with significant morbidity and potential mortality. Correct diagnosis with identification of all offending allergens is an absolute prerequisite for appropriate and potentially life-saving preventive measures or the initiation of allergen-specific immunotherapy. The diagnostic algorithm for IgE-mediated allergy involves a thorough clinical history that is documented by the demonstration of allergen-specific IgE (specific IgE) antibody by either *in vivo* skin testing (prick/intradermal) or a serologic assay. Although these tests can provide useful information, they do not demonstrate an absolute predictive value

and the results are not always unequivocal. In these cases, where there exists a discrepancy between history and results from quantification of sIgE and/or skin tests, additional tests such as mediator release assays or (double-blind placebo-controlled) challenge tests have long been used to establish correct diagnosis.

During the last decade two novel *in vitro* assays have entered the allergy diagnostics arsenal, that is, component-resolved allergy diagnosis (CRD) and the basophil activation test by flow cytometry.

Past

IgE was discovered in 1967 as the ‘reagine’ responsible for so-called type I hypersensitivity reaction [1,2]. Approximately 5 years later, the first *in vitro* assay for sIgE, called the radio allergosorbent test (RAST) was commercialized. The original RAST, was configured as a cyanogen-bromide activated paper disc on which different native allergen extracts were covalently coupled. In this assay sIgE that complexes with the coupled allergen is quantified with radioiodinated polyclonal antihuman IgE using a γ -counter [3]. Today, traditional RAST has been almost abandoned and replaced by several automated and technically optimized immunoassays that generally display excellent analytic sensitivity, precision, reproducibility (intra- and inter-assay) and linearity [4,5]. However, some issues should be kept in mind for correct interpretation of the results. First, none of the currently (commercially) available sIgE assays demonstrates an absolute predictive value and a positive sIgE result should be interpreted with prudence as it is not a *de facto*

indication of the presence of allergic disease but can merely reflect (cross)sensitization. For example, ubiquitous structures, such as profilins and cross-reactive carbohydrate determinants (CCD), present on glycoproteins of plants and hymenoptera venom have been frequently shown to elicit positive, but clinically irrelevant, serology with a broad spectrum of cross-reactivity involving pollen, *Hevea* latex, plant food and venoms [6]. Alternatively, a negative sIgE result by no means excludes allergy. Second, it is important to realize that results and experience obtained with different (commercially available) assays are not interchangeable (for a review, see [4]). For example, data on levels of sIgE predictive of the outcome of food challenges [7,8] or the presence of more severe disease [9] should be considered as strictly specific for the assay that was studied. These concerns relate to the fact that traditional sIgE assays use heterogeneous (protein) extract-based allergens from different sources and it is anticipated that the serological allergy diagnosis should benefit from the introduction of better standardized and well-characterized allergen components and functional assays, such as flow-assisted basophil activation tests, that enable assessment of the ability of an extract to trigger the effector cells of the allergic reaction.

Present

During the last decade, two new *in vitro* techniques have entered the field of allergy diagnosis: CRD and the basophil activation test.

Component-resolved (allergy) diagnosis

Recent advances in biochemistry and molecular biology enabled the characterization, cloning and recombinant production of most relevant allergen proteins and epitope-emulating peptides, allowing detection and quantification of sIgE antibodies to these proteins or sequential epitopes; this process is known as CRD. In contrast to traditional sIgE assays, CRD does not rely upon whole extract preparations from native allergens but on quantification of sIgE antibodies to single protein or polypeptide components purified from natural sources or obtained by recombinant techniques. There are various databases on allergen components and protein families, such as the official International Union of Immunological Societies (IUIS) allergen nomenclature database [101] and the Allfam allergen database grouping allergens into protein (sub)families [102].

The principles, applications and pitfalls of CRD that can be performed by traditional singleplexed assays as well as by microarray technology are extensively reviewed elsewhere [10–12]. Briefly, CRD involves specific (unique) marker components and/or substructures to study the genuine sensitization of patients towards a particular allergen and the presence of sIgE antibodies to cross-reactive components or epitopes, such as CCD or ubiquitous pan-allergens that point to cross-sensitization to several even unrelated allergen sources. This approach not only enables the establishment of individual primary and crossreactive sensitization profiles (revealing which parts of allergen[s] are recognized by an individual patient), but can also help to discriminate between genuine allergy and sensitization. Furthermore, CRD allows an individual risk assessment of severity and possibly the prediction

of persistence, facilitates the selection of patients for allergen-specific immunotherapy, and may eventually help in monitoring the immunological effects of a treatment. Nevertheless, further clinical validation is mandatory before the technique can enter mainstream application. By no means can CRD currently be considered as a substitute to traditional sIgE assays.

Most recently, it has emerged that the exploration of the IgE response to particular allergens can extend beyond the level of components and focus on epitopes of those proteins. From these studies, it appears that analysis of epitope binding patterns might further add to diagnosis and enable better estimation of severity, phenotypic expression and prediction of natural history (for a review, see [13]).

Basophil activation tests

Basophil mediator release assays have predominantly focused on histamine and sulphidoleukotriene release. However, the time- and cost-consuming two-step procedure consisting of cell isolation and incubation and quantification of (labile) mediators has hampered their application [14]. Two decades ago, the discovery of the basophil activation markers CD63 (gp53) [15], and subsequently of CD203c (ENPP-3) [16], induced the development of a novel technique to analyze and quantify allergen-specific *in vitro* activation of basophils (basophil activation test [BAT]) [17]. Briefly, the BAT relies on flow cytometric identification and quantification of alterations of various specific activation markers on the surface membrane or inside the cells. These changes can be detected and quantified on a single-cell level using specific monoclonal antibodies coupled to particular fluorochromes. Practically, basophils are characterized by specific markers such as CCR3⁺/CD3⁺, CD123⁺/HLA-DR⁺ or IgE⁺/CD203c⁺. Of these markers, only CD203c is lineage specific. Subsequently, in a second step, the upregulation or appearance of specific surface activation markers, such as CD63 and/or CD203c, is quantified. Activation of basophils can also be quantified by means of intracellular molecules, such as phosphorylation of MAPK (p38MAPK) [18]. At present, BAT has proven to be a rapid and reliable diagnostic method that allows the diagnosis of allergy to various inhalant allergens (pollen, house dust mite and cat), food (including pollen-related food allergies), natural rubber latex, hymenoptera venom and certain drugs (mostly curares and β -lactam antibiotics). In addition, the technique has proven to be useful in non-IgE-mediated reactions, such as hypersensitivity to drugs as well as detection of IgG anti-Fc ϵ RI or anti-IgE autoantibodies in chronic autoimmune urticaria.

Finally, our department has recently shown that intracellular histamine and its release by *in vitro* activated basophils can successfully be analyzed by multicolor flow cytometry (HistaFlow) applying an enzyme-affinity fluorochrome method, based on the affinity of the histaminase diamine oxidase for its substrate [19].

Future

It is clear that component-resolved diagnosis and flow-assisted analysis of *in vitro*-activated basophils provide the physician with novel promising diagnostic approaches. Particularly in the field of

food allergy, CRD constitutes a key advance with better estimation of severity, phenotypic expression and improved insights into prognosis. Most recently, encouraging advances have been made in epitope analyses and it seems justified to anticipate that epitope resolved allergy diagnosis will further improve management of the allergic patient by capturing data that are inaccessible for other techniques. On the other hand, multiple studies in immediate drug allergy have clearly emphasized the potential of flow-assisted basophil activation tests. Preliminary data indicate that the analyses of new activation markers should enable broadening of knowledge on

the pathophysiology of pseudoallergic (non-IgE-mediated) drug allergies and add to the diagnosis of these reactions.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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Websites

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- Allfam allergen database. www.meduniwien.ac.at/allergens/allfam