

REVIEW ARTICLE

Use of a rat basophil leukemia (RBL) cell-based immunological assay for allergen identification, clinical diagnosis of allergy, and identification of anti-allergy agents for use in immunotherapy

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Abstract

Food allergy is an important public health problem that affects an estimated 8% of young children and 2% of adults. With an increasing interest in genetically-engineered foods, there is a growing need for development of sensitive and specific tests to evaluate potential allergenicity of foods and novel proteins as well as to determine allergic responses to ensure consumer safety. This review covers progress made in the field of development of cell models, specifically that involving a rat basophil leukemia (RBL) cell-based immunoassay, for use in allergen identification, diagnosis, and immunotherapy. The RBL assay has been extensively employed for determining biologically relevant cross-reactivities of food proteins, assessing the effect of processing on the allergenicity of food proteins, diagnosing allergic responses to whole-food products, and identifying anti-allergy food compounds. From the review of the literature, one might conclude the RBL cell-based assay is a better test system when compared to wild-type mast cell and basophil model systems for use in allergen identification, diagnosis, and analyses of potential immunotherapeutics. However, it is important to emphasize that this assay will only be able to identify those allergens to which the human has already been exposed, but will not identify a truly novel allergen, i.e. one that has never been encountered as in its preferred (humanized) configuration.

Keywords

Allergen identification, cell models, clinical diagnosis, immunotherapy, rat basophil leukemia (RBL) cells

History

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Introduction

Food allergy, defined as an immune-mediated adverse response to food proteins, is an important public health problem that affects adults and children, and appears to be increasing in prevalence. In westernized countries, food allergy affects an estimated 8% of young children and 2% of adults (Cianferoni, 2014). Food-induced allergic reactions may be responsible for different clinical symptoms that can affect the skin, and the digestive and respiratory tracts (Dupont, 2011). Despite the risk of severe allergic reactions and even death, there is no curative treatment for food allergy, and strict avoidance of the offending food remains the only effective measure to prevent allergic reactions. This strategy is not optimal as there is still the risk of unintentional ingestion due to mislabeling of pre-packaged foods, allergen cross-contact during manufacture, or consuming foods in restaurants and catering (Vogel et al., 2006). Besides, with an increasing interest in genetically engineered foods, the potential allergenicity of newly introduced proteins has become an important safety evaluation issue. Therefore, sensitive and specific tests are required for identification of allergenic proteins in food to ensure consumer safety and for determination of allergic responses.

Double-blind placebo-controlled food challenges (DBPCFC) have been considered the gold standard for evaluation of food allergy; however, these are costly and potentially dangerous since they can trigger severe reactions in some subjects (Rancé et al., 2002). Consequently, reliable safe screening tests are still needed to assess reactivity to food allergens. Skin prick tests (SPT) provide a rapid means to detect sensitization for food-induced allergic disorders. Several different methods of interpreting SPT are employed, but their value in accurately predicting food allergy reactions remain uncertain (Knight et al., 2006; Serup & Staberg 1985; Sussmang et al., 1982). Among them, wheal size is the most commonly evaluated endpoint. The results of food allergen SPT are generally recorded as positive when the mean wheal diameter is ≥ 3 mm compared against the negative control. However, this method may not sufficiently reflect the actual size of those wheals with irregular shapes (Eigenmann & Sampson, 1998). More importantly, SPT cannot be predictive of symptomatic IgE-mediated food allergy (Ocmant et al., 2009).

In addition to *in vivo* tests, *in vitro* methods such as enzyme immunoassay or western blotting have been commonly used in determining the allergenic potential of food proteins and in clinical diagnosis of food allergy. Immunochemical methods depend on IgE that is present in allergen-specific polyclonal animal sera or human patient sera to bind to molecules bound to solid matrices like cellulose (enzyme allergosorbent test [EAST], Immuno-CAPs) or nitrocellulose (Western blotting). This binding to the matrix may change the structural integrity of the allergens and, thus, destroy the epitopes responsible for inducing allergy

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in vivo (Palmera et al., 2005; Vogel et al., 2005). Further, the results of immunochemical methods that are based on allergen-specific IgE are susceptible to a presence of IgG of the same specificity (Kadooka et al., 2000). In measuring specific IgE levels in sera by direct ELISA, allergens immobilized on ELISA microplates are bound largely by specific IgG that exist in sera at concentrations that are $\approx 10\,000$ -times that of IgE. Thus, specific IgE cannot bind sufficiently to the immobilized allergens because of this phenomenon of competition (Kadooka et al., 2000).

Mechanistically, binding of food allergens by specific IgE on effector cells, such as basophils and mast cells, leads to mediator release (i.e. histamine, leukotrienes), causing a variety of symptoms as mentioned above (Wang & Sampson, 2009). However, immuno-chemical methods only detect binding of IgE to allergens, and not the potential of the allergens to elicit an IgE-mediated allergic reaction, as is the case with *in vivo* tests. Additionally, our previous study demonstrated little correlation between serum IgE antibody levels and severity of clinical symptoms, an outcome consistent with findings of other investigators (i.e. Blanco et al., 1998; van der Veen et al., 1997; Sharnan et al., 2001; Sun et al., 2013; van Ree & Aalberse 1999).

To overcome these problems, researchers have investigated the use of cell models to identify potential allergens in a toxicological/safety context and determine the allergic response to a food product in the clinic. The identification of potential allergens in a toxicological/safety context is based on two steps: generation of murine sensitized serum and the performance of an allergen dose-dependent mediator release assay from rat or murine basophils/mast cells that are passively sensitized with murine sensitized serum and stimulated with the corresponding allergen (Hoffmann et al., 1999). Furthermore, for determining allergen susceptibility of a patient, human or humanized basophils/mast cells are often sensitized with heat-inactivated serum from allergic patients and subsequently stimulated with a panel of allergens. This gives rise to a high level of mediator release ($>30\%$ of cellular mediators) (Rashid et al., 2012) as well as up-regulation of CD63 or CD203c on basophil membranes (Ocmant et al., 2009).

This review paper presents in details an up-to-date review of the progress made in the development of cell models as well as the applications of a RBL cell-based immunoassay that could be used for allergen identification, diagnosis of allergy, and identification of potential substances that could be used in immunotherapeutics against food allergy. However, the RBL cell-based immunoassay also has the limitation that this assay will not identify a truly novel allergen, i.e. one that has never been encountered as in its preferred (humanized) configuration.

Current cell models

Food-induced allergic reactions are mediated by mediator release (i.e. histamine, leukotrienes) in response to the allergens cross-linking of specific IgE bounding to $F_{c\epsilon}RI$ on mast cells or basophils (Wang & Sampson, 2009). Therefore, an *in vitro* cell-based immunological assay, based on the activation and mediator release of effector cells, is of considerable interest to study the biological activity of food proteins. Various cell models have been proposed over the years in an effort to establish a cell model used for allergen identification and clinical diagnosis. Table 1 summarizes the main characteristics of an ideal cell model.

Mast cell model

Mast cells were discovered by Ehrlich (1877). Mature mast cells exist exclusively in tissues and particularly in regions located at interfaces with the external environment, such as skin, lungs and mucosal surfaces (Gibbs et al., 1997). Mast cells play a pivotal

Table 1. Main characteristics of an ideal cell model.

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| <ul style="list-style-type: none"> • Release mediators after IgE/antigen sensitization • Easily available and reproducible between laboratories • Specific for discriminating potent food allergens from allergenic to non-allergenic proteins • Sensitive for distinguishing threshold beyond which significant allergic response would be predicted |
|---|

role in initiating immediate allergic reactions. Cross-linking of IgE-bound $F_{c\epsilon}RI$ by allergens resulting in a rapid release of pre-formed and newly-synthesized mediators (purple material in Figure 1A). Preformed mediators such as histamine, heparin, chemotactic factors, tryptases, and chymases are stored in granules and secreted via exocytosis; newly-synthesized mediators such as prostaglandin D_2 , leukotrienes C_4 and D_4 , and platelet activating factor are produced and secreted after cell stimulation (Church et al., 2003). These mediators give rise to allergic symptoms, ranging in severity from simple urticaria to anaphylactic shock and death.

Mast cell isolation from small animals (e.g. rats, mice) via peritoneal lavage is simpler compared with those in other tissues. Histamine release assay of the peritoneal mast cells (PMC) has proven to be sensitive and specific for allergen identification (Guo et al., 2009). PMC of sensitized mice were incubated with corresponding allergen, presenting high mediator release. Moreover, when sensitized mast cells encountered the non-corresponding allergens, the degranulation was $<20\%$ in general, a level much lower than specific degranulation (Guo et al., 2009). However, some disadvantages limit the use of PMC as a suitable cell model to assess the allergenicity of food proteins. First, the cells need to be purified, which can hamper their ability to react to stimuli (Coutts et al., 1980). Second, it is impossible to maintain these cells in primary cultures over prolonged periods of time (Horigome et al., 1994).

Human basophil model

Basophils are granulocytes that develop from $CD34^+$ pluripotent progenitor stem cells that differentiate and mature in the bone marrow. They then circulate in the periphery where they represent $<1\%$ of the white blood cell population (Ebo et al., 2008; Passante & Frankish, 2009). Like mast cells, basophils are also key effector cells in allergic disorders and secrete different granule-stored mediators responsible for allergies and other inflammatory diseases. Actually, on challenge with specific allergens, basophils not only secrete quantifiable bioactive mediators but also up-regulate expression of different markers that can be detected efficiently by flow cytometry using specific monoclonal antibodies (Ebo et al., 2006). The corresponding methods are basophil mediator release assay and basophil activation test (BAT).

Generally, functional *in vitro* tests have focused on basophil mediator release assays such as basophil histamine release (BHR) (Crockard & Ennis, 2001; Mittag et al., 2004; Toda et al., 2011). Superior diagnostic specificity (82%) was observed in BHR compared to specific IgE (24%) and SPT (32%). Assay sensitivity, however, was less impressive, i.e. BHR (53%); specific IgE (82%) and SPT (82%) (Crockard & Ennis, 2001). However, histamine is released by basophils a few minutes after the reaction levels are maximal in peripheral blood, and it is rapidly metabolized to *N*-methyl-histamine that is eliminated via the urine (Mayorga et al., 2010). Because of this, the measurement of histamine in peripheral blood is difficult and restricts its use in clinical applications.

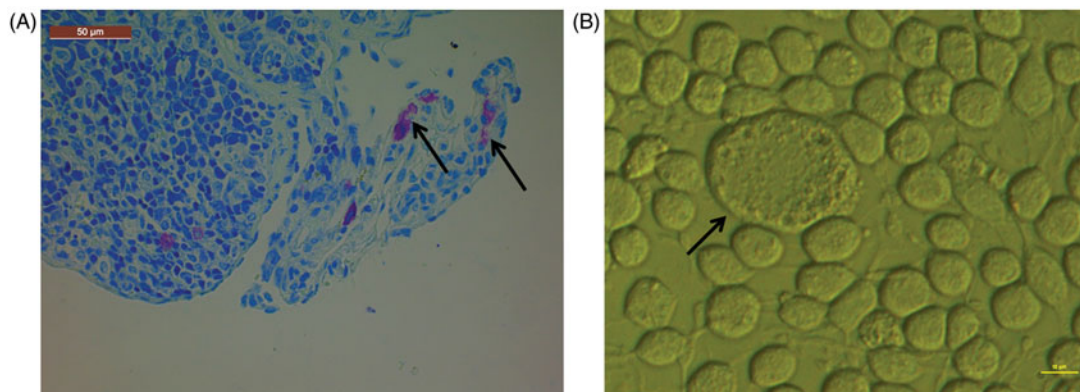


Figure 1. Pictures of mediator release of mouse mast cells and RBL cells (arrows). (A) Mast cells in small intestine of Balb/c mice stained with toluidine blue for microscopic examination (Type DM 1000, Leica, Geneva, Switzerland). (B) RBL cells examined by fluorescent inverted microscope (Type TH4-200, Olympus, Tokyo, Japan).

The discovery that CD63 was up-regulated concomitantly with basophil mediator release led to the development of a flow cytometric technique to analyze and quantify allergen-specific *in vitro* activation of peripheral blood basophils (Ebo et al., 2006). Subsequently, Sainte-Laudy et al. (1994, 1996) and Sabbah et al. (1997, 1998) developed a basophil activation test (BAT) based on anti-CD63 to evaluate the activation of these cells by flow cytometry. In the subsequent research performed to optimize this technique, other cellular markers such as the marker of activation of basophils CD203c were found. Since the mid-1990s, BAT based on CD63 and CD203c has proven reliable for the diagnosis of IgE-mediated allergies (Buhning et al., 2004; Hauswirth et al., 2002; Kahlert et al., 2003; Mayorga et al., 2010; Ocmant et al., 2009). As compared with routine diagnostic tests specific IgE or SPT, BAT was less sensitive, particularly when including non-responders, but was more specific, achieving at least 95% specificity (Ocmant et al., 2009). However, BAT, rather than specific IgE or SPT, could provide an additional tool to discriminate between clinically relevant food-specific IgE versus irrelevant IgE responses. The use of BAT might be justified when SPT or specific IgE determinations are not feasible or give equivocal results with regard to the clinical history (Ocmant et al., 2009). Nevertheless, widespread use of this method in clinical laboratories is prevented by technical limitations. First among these is the processing of blood samples immediately after collection. Another is sample type (i.e. whole blood versus separated cells). Although whole blood may better reflect *in vivo* physiologic status, other factors could interfere with the assay. Cell separation (which would avoid such interference) could result in a loss of basophils and a possibility that handling might result in non-specific activation (Mayorga et al., 2010).

Rat basophil leukemia (RBL) cell model

Mast cells obtained by peritoneal lavage and basophils obtained from peripheral blood suffer from a few disadvantages that limit their widespread use. One is that they each need to be purified. More importantly, it is impossible to maintain them in primary culture over prolonged periods of time (Coutts et al., 1980; Horigome et al., 1994). Keeping in mind these problems, the availability of a permanently growing rat basophilic leukemia (RBL) cell line, presenting some characteristics of both mast cells and basophils, has kindled the enthusiasm of researchers.

The most commonly used cell line, designated RBL-2H3, was cloned by a limited dilution technique from leukemia cells isolated from rats treated with β -chloroethylamine (Siraganian et al., 1982). As shown in Figure 1(B), RBL-2H3 cells - like mast cells and basophils - respond by undergoing degranulation with

release of a range of pre-formed and newly-synthesized mediators that evoke a potent allergic response, following cross-linking by allergens of their IgE-bound $F_{c\epsilon}RI$. RBL-2H3 cell degranulation can be monitored by following release of two mediators, β -hexosaminidase and histamine; release of each is closely parallel, time-wise (Passante et al., 2009). Compared with histamine, β -hexosaminidase is more suitable as a biomarker of RBL-2H3 degranulation due to its easy rapid detection (Passante & Frankish, 2009).

In view of their similar functional characteristics to mast cells, RBL-2H3 cells sensitized with allergen-specific murine IgE have been widely used by many groups for the determination of biological activity of allergen extracts and allergens (Ballmer-Weber et al., 2002; Hoffmann et al., 1997, 1999; Kaul et al., 2007). Because of the species restriction of the $F_{c\epsilon}RI$ in RBL-2H3 cells, the assay requires murine IgE. Doubtless, the most crucial point of this assay is murine IgE used for passive sensitization of RBL-2H3 cells. The immunization schedule based on repeated applications of low doses of allergens seems to be generally suitable to induce allergen-specific IgE in Balb/c mice (Hoffmann et al., 1997, 1999; Kaul et al., 2007). Most major allergens can be recognized by both murine and human sera (Kaul et al., 2007; Knippels et al., 2000); however, to some extent, the recognized allergens may differ (Kaul et al., 2007). For example, the major allergens Gal d 1, Gal d 2, Gal d 3, Ara h 1, the Ara h 3/4 complex, and Bos d 5, and the minor allergens Gal d 4 and Bos d 8, were detected by both human and murine sera. In contrast, Ara h 2 was not detected by murine sera (Kaul et al., 2007; Knippels et al., 2000). Thus, the RBL cell-based immunological assay based on murine IgE may not be fully identical to those based on human IgE.

To resolve this, RBL-2H3 cells were transfected with cDNA coding for human high-affinity IgE receptor ($F_{c\epsilon}RI$) chains (Dibbern et al., 2003; Gilfillan et al., 1992; Kaul et al., 2007; Ladics et al., 2008; Takagi et al., 2003; Taudou et al., 1993; Vogel et al., 2005). The human $F_{c\epsilon}RI$ allowed these cells to bind IgE from sera of allergic subjects and subsequently be activated in allergen-specific manners. Mediator release by humanized RBL cells provided an appropriate cell-based assay to measure the biological activity of allergens *in vitro* and, over time, one that proved to be sensitive, specific, and reproducible (Blanc et al., 2009; Dibbern et al., 2003; Kaul et al., 2007; Untersmayr et al., 2005; Vogel et al., 2006). Humanized RBL cells, in combination with human serum pool, might then represent a preferred test system. On the other hand, murine sera may be beneficial in cases of "rare" allergens (such as mutated recombinant allergens) for which human sera are difficult to obtain. Although allergen-binding patterns of murine and human sera were not fully

Table 2. Summary of cell models for allergenicity evaluation and clinical diagnosis.

Cell models	Protocol	Strengths	Weaknesses	References
Murine peritoneal mast cells	Histamine release	Sensitive, specific	Requires purification, semi-permanent cultivation	Guo et al. (2009)
Human basophils	Histamine release	Specific	Measure of histamine is difficult	Crockard & Ennis (2001); Mittag et al. (2004); Toda et al. (2011)
Human basophils	Basophil activation	Specific	Consecutive blood sampling to obtain the basophils	Sainte-Laudy et al. (1994, 1996); Sabbah et al. (1997, 1998); Hauswirth et al. (2002); Kahlert et al. (2003); Buhning et al. (2004); Ocmant et al. (2009); Mayorga et al. (2010)
RBL cells	β -hexosaminidase release assay	Easily available; permanent cultivation; sensitive, specific and reproducible	Allergen-binding patterns of mouse and human sera might not be fully identical	Hoffmann et al. (1997, 1999); Ballmer-Weber et al. (2002); Kaul et al. (2007)
Humanized RBL cells	β -hexosaminidase release assay	Easily available; permanent cultivation; sensitive, specific and reproducible	Rare allergens for which human sera are difficult to obtain	Gilfillan et al. (1992); Takagi et al. (2003); Taudou et al. (1993); Dibbern et al. (2003); Untersmayr et al. (2005); Vogel et al. (2005, 2006); Kaul et al. (2007); Ladics et al. (2008); Blanc et al. (2009)

identical, both mouse and human systems revealed very similar potency data and complemented each other; thus, a wide variety of allergens could be analyzed (Kaul et al., 2007).

A number of serum factors (i.e. IgE-related) as well as allergen factors have been suggested as important for inducing mediator release with RBL cell lines. The relationship between immunochemical parameters of IgE and functional degranulation responses in these transfectants has been addressed. Dibbern et al. (2003) showed that the absolute amount of peanut-specific IgE is an important determinant of the ability of a serum to effectively sensitize cells for allergen-dependent degranulation ($r=0.95$; $p<0.001$), but that the amount of total IgE was a relatively poor predictor. Conversely, Marchand et al. (2003) indicated no correlation ($r=0.27$) between the proportion of bound allergen-specific IgE and strength of the degranulation response after sensitization with serum IgE. However, a significant correlation ($r=0.97$) was found for purified monoclonal IgE. Therefore, RBL cell activation mediated through serum IgE is a result of complex relationships not only dependent on allergen-specific IgE content, but also the capacity to efficiently sensitize cells and trigger signaling responses that lead to degranulation. Further, some serum factors may hamper binding of IgE to $F_{c\epsilon}RI$ expressed on the cell membrane and adverse effects on the cells occur (Dibbern et al., 2003; Marchand et al., 2003; Vogel et al., 2006). Those factors and how they may act on cells to interfere with the assay have not yet been specifically identified. Nevertheless, the IgE-immunopurification of sera was a simple, rapid, and efficient treatment to remove interfering factors with regard to background release, sensitivity, and reproducibility of the assay (Blanc et al., 2009). In addition, the optimum dilution range for the serum of each allergic subject may need to be determined. Incubation with undiluted serum or $>20\%$ serum was found to cause cytotoxic effects (i.e. decrease viability) in the RBL cells (Ladics et al., 2008; Marchand et al., 2003). This toxicity appears to be due to naturally occurring xenoreactive antibodies (Palmera et al., 2005; Schuurman et al., 2003). Further, the optimal concentration for allergens or allergen extracts may also be important for inducing IgE-mediated responses with such cell lines. High concentrations of allergens or allergen extracts might induce a non-IgE-mediated release on stimulation of non-sensitized RBL cells (without added human serum); this might be due to a presence of protease, endotoxin, or (1,3)- β -D-glucans

(Nowak-Wegrzyn et al., 2009; Trivedi et al., 2003). Thus, it should be required for any RBL cell-based immunological assay to obtain the optimal configuration of experimental parameters before the assay could be applied to allergen identification, or use in clinical diagnosis and/or immunotherapy.

A summary of cell models found useful for allergenicity evaluation and clinical diagnosis has been provided in Table 2.

Applications of an RBL cell-based immunological assay in allergen identification, clinical diagnosis, and immunotherapy

Determining biologically relevant cross-reactivity of food proteins

According to both FAO/WHO (2001) recommendations and Codex (2003) guidelines for the assessment of allergenic potential of novel food proteins, potential cross-reactivity of a novel biotechnology protein with a known allergen needs to be considered when their sequences are $>35\%$ identical over an 80-amino-acid window. As a rule, serum IgE testing is employed to investigate the IgE cross-reactivity of proteins (Goodman et al., 2008); however, they are non-functional immunochemical methods and cannot measure the biological reactivity of proteins. Therefore, if serum IgE binding is demonstrated, the biologically relevant cross-reactivity could also be tested by an RBL cell-based assay.

The latter assay may also serve as a useful tool for investigating whether or not a biologically relevant cross-reactivity could exist between two known allergens. In general, strong biologically relevant cross-reactivity is expected between highly homologous proteins. In the RBL assay, however, this was not the case. For example, despite a high sequence identity (87–91%) between bovine and caprine β -caseins, no biologically relevant cross-reactivity was noted with the RBL cell-based assay (Hazebrouck et al., 2014). Thus, the RBL cell-based assay may differentiate between proteins that have been shown to have homology from sequence data, but in fact do not result in the clinically observed cross-allergenicity. Further, the analysis of the biologically relevant cross-reactivity of food proteins by an RBL cell-based immunological assay may also be useful for identifying biological characteristics of allergens (Scheurer et al., 1999;

Sordet et al., 2009) and in the clinical diagnosis of food allergy (Jarvinen et al., 2011).

Assessing the effect of processing on allergenicity of food proteins

The effect of processing on food allergens is of major interest for individuals who need to avoid all food preparations containing active allergens. Food processing may destroy existing IgE-binding epitopes on a protein or conversely generate (neoallergen formation) new ones as a result of changes in protein conformation (Mills et al., 2009; Sathe et al., 2005). These changes may affect allergic sensitization to food proteins and the elicitation of allergic reactions in sensitized individuals. Taken together, these clearly could impact on the allergenic potential of food proteins. The effect of processing on allergenicity of proteins has been assessed in the past by determining IgE-binding capacity using immunoassays/immunoblotting (Blanc et al., 2011; Ehn et al., 2005; Jimenez-Saiz et al., 2011; Sancho et al., 2005). In a study by Vissers et al. (2011), a very significant decrease in IgE-binding capacity was noted with heated Ara h 1, but its capacity to elicit mediator release from RBL cells was significantly enhanced. It is important to state that only using IgE-binding tests to study the effect of food processing is insufficient to fully assess the degree of allergenicity of the (modified) protein; the impact of processing on cross-linking and cell degranulation activity must also be considered. This is the case as processing-induced modifications are likely to affect availability of multiple epitopes required for effective cross-linking of cell surface-bound IgE. Therefore, a RBL cell-based immunoassay coupled with IgE binding assays would be an optimal approach to investigate allergenic potentials of food proteins and to permit comparisons of the allergenicity of foods before and after processing.

Diagnosing an allergic response to a whole-food product

Contrary to the perception that, when diagnosing an allergy in a subject, purified allergens or allergen extracts should be used, an RBL cell-based immunoassay may provide information on the ability of a whole-food product to elicit an allergic reaction (van Escha et al., 2011). This would be especially useful when testing for allergies from food products that contain a great number of components with multiple allergy-inducing epitopes. Such an assay would be appropriate for critically ill subjects in whom it is not (yet) known whether or not there was an allergic response to food allergens. An allergic reaction to a food product in these patients may lead to very severe illness or even death. Hence, determining if these patients are allergic to a whole-food product or determining which product causes an allergic response is critical. In addition, this assay is also particularly beneficial for the examination of infants that do not have a documented history of food allergy. This would be the case for testing potential allergic responses to infant milk and tube-fed nutritional formulas (Tregoeat & Garssen, 2011). It might be said that a subject shows an allergic response if the degranulation is at least 30% (preferably at least 50%) of the degranulation caused by use of a positive control, i.e. usually purified IgE, in the same assay. It is preferable to say that a subject shows an allergic response if their degranulation is significantly greater than that measured in the same degranulation assay due to exposure to an appropriate negative control sample, i.e. usually serum of non-allergic subject or a pool of sera from non-allergic subjects (Tregoeat & Garssen, 2011).

Identification of anti-allergy compounds in foods

In recent studies, it was reported that various dietary natural products can inhibit degranulation via suppression of signaling

pathways, suggesting their potential use in allergy therapy (Chen et al., 2012; Chung et al., 2013; Han et al., 2011; Ishida et al., 2013; Kobayashi & Tanabe, 2006; Kuba-Miyara et al., 2012; Zhang et al., 2012). In allergic reactions, signal transduction is triggered by the allergen cross-linking mast-cell surface allergen-specific IgE-bound high-affinity receptors ($F_{c\epsilon}RI$). This initiates a signaling phosphorylation cascade composed of two main pathways: the principle (Lyn-Syk-LAT-PLC γ_1) and complementary (amplification) (Fyn-Gab2-PI3K) pathways. After the phosphorylation cascade, release of Ca^{2+} from intracellular stores, followed by extracellular Ca^{2+} influx, occurs. These events lead eventually to conformational changes in the cytoskeleton that in turn induce degranulation and release of mediators like histamine, β -hexosaminidase, and pro-inflammatory cytokines like interleukin (IL)-4 and tumor necrosis factor (TNF)- α (Gilfillan & Tkaczuk, 2006; Kuba-Miyara et al., 2012).

Various food compounds impart anti-allergy effects via different inhibitory mechanisms of mast cell degranulation. In RBL-2H3 cells, polyphenolic compounds from grape seed extract blocked IgE-induced degranulation partly through the suppression on $F_{c\epsilon}RI$ expression and decreased Ca^{2+} uptake (Chen et al., 2012). Suppression of the phosphorylation of Syk and PLC γ_1 in the principle signaling pathway might be involved in the attenuated degranulation of these cells by okicamelliaside from *Camellia japonica* leaves (Kuba-Miyara et al., 2012). Flavonoids from *Citrus unshiu* inhibited RBL-2H3 cell degranulation, in part, via a suppression of PI3K activity in the complementary signaling pathway (Kobayashi & Tanabe, 2006). Thus, an RBL cell-based immunoassay could be a convenient tool to screen for potential anti-allergy food compounds that could then be used for immunotherapies against food allergies.

Conclusion

It is evident that considerable progress has been made in developing cell models used for allergen identification, clinical diagnosis of food allergy, and for analyses of potential immunotherapeutics. We conclude that, with regard to those endpoints and culture characteristics, a RBL cell-based assay is a better test system when compared to wild-type mast cell and basophil model systems. However, it is important to emphasize that this assay will only be able to identify those allergens to which the human has already been exposed, but will not identify a truly novel allergen, i.e. one that has never been encountered as in its preferred (humanized) configuration. Thus, while the RBL system is optimal for many of the above-noted reasons, only through continued improvements in technology and an increased understanding of allergic mechanisms will new testing methods even better than the RBL immunoassay be developed that will be able to identify truly novel allergens.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This work was supported by National Natural Science Foundation of China (NO. 81273077).

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