

Use of Humanized Rat Basophilic Leukemia Reporter Cell Lines as a Diagnostic Tool for Detection of Allergen-Specific IgE in Allergic Patients: Time for a Reappraisal?

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Abstract The interaction between allergens and specific IgE is at the heart of the allergic response and as such lies at the center of techniques used for diagnosis of allergic sensitization. Although serological tests are available, *in vivo* tests such as double-blind placebo-controlled food challenges (DBPCFC) and skin prick test (SPT) associated to the patients' clinical history are still the main guides to clinicians in many practices around the world. More recently, complex protein arrays and basophil activation tests, requiring only small amounts of whole blood, have been developed and refined, but are yet to enter clinical practice. Similarly, the use of rat basophilic leukemia (RBL) cell lines for detection of allergen-specific IgE has been made possible by stable transfection of the human FcεRI α chain into this cell line more than 20 years ago, but has not found widespread acceptance among clinicians. Here, we review the perceived limitations of diagnostic applications of humanized RBL systems. Furthermore, we illustrate how the introduction of reporter genes into humanized RBL cells is able to overcome most of these limitations, and has the potential to become a new powerful tool to complement the armamentarium of allergists. A demonstration of the usefulness of humanized RBL reporter systems for elucidation of complex IgE sensitization patterns against

wheat proteins and a section on the use of fluorescence-based reporter systems in combination with allergen arrays close the review.

Keywords RBL · RS-ATL8 · NFAT-DsRed · IgE · Allergic sensitization · Allergy diagnosis

Introduction

The crosslinking of specific IgE (sIgE) bound to the high affinity receptor FcεRI on mast cells and basophils in the presence of cognate antigen results in fast degranulation and release of preformed as well as *de novo* synthesized mediators; these are responsible for most of the well-known allergic symptoms [1]. Therefore, the measurement of sIgE levels in the blood of individuals with suspected allergy is at the center of allergy diagnosis. These measurements however, despite being sensitive, are frequently unconvincing to a reliable diagnosis in isolation, particularly in the context of food allergy. In essence, the presence of sIgE directed against certain allergens (sensitization) is not the equivalent of clinical symptoms (allergy) to a specific source of allergens [2, 3]. In addition to a detailed allergen-focused clinical history and serological sIgE tests, cellular tests have also been frequently used in the identification of suspected allergens. The most commonly used cellular assay is based on detection of activated mast cells after intradermal introduction of test samples containing the suspected allergens (skin prick test, SPT). SPT has a long pedigree going back to the original work of Charles H. Blackley in the second half of the nineteenth century [4]. SPT testing has the potential to remove some of the uncertainty associated with sIgE testing, by adding a cellular dimension able to distinguish between clinically relevant sensitization to multivalent allergens and to substances deprived of ability to

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activate mast cells and basophils, such as those containing cross-reactive carbohydrate determinants (CCDs). As the final readout for SPT is indirect (measurement of the wheal and flare reaction caused by the release of histamine in the skin), among others it is affected by the use of medications targeting histamine receptors, and due to the residual risk of complications, it needs to be carried out in specialized units. A safer alternative is represented by the basophil activation test (BAT), which uses small amounts of blood and is not affected by the use of anti-histamines or other medications affecting SPTs. BAT has recently been successfully used in the discrimination of peanut-allergic and peanut-sensitized children where differences in IgE levels did not reflect the clinical phenotype. Specific IgG4 has been shown to inhibit basophil activation in these cases [5•]. However, despite being investigated intensively, the basophil activation test is still awaiting FDA approval, and due to the requirement for specialized equipment, it is likely to remain confined to specialized research units.

In addition to SPTs and BATs, a third cell-based diagnostic technology for the assessment of sensitization has been put forward by several groups: the use of humanized rat basophilic leukemia (RBL) cell lines sensitized with patient serum. This technology has the potential to combine the advantages of sIgE testing (high sensitivity, mid- to high-throughput capability) with the clinical relevance of using a biological readout without the expensive and dedicated specialized equipment. It is postulated here, but still in need of experimental further verification, that a cellular readout, in the form of humanized RBL or high affinity IgE-receptor expressing human cell lines such as LAD2 or ROSA (KIT WT) [6], will be able to substantially improve the diagnostic of clinical symptomatic (allergy) rather than mere sensitization (IgE levels only) thereby reducing the occurrence of false positive results.

However due to a set of perceived or actual limitations, these cells are not generally in use for diagnostic purposes. Here, we review the progressive development and use of humanized RBL cells, the initial perceived limitations of these systems, and the more recent developments ultimately leading to robust and sensitive reporter systems which have the potential to develop into an essential component of tomorrow's allergy diagnostic armamentarium.

Use of Humanized RBL Non-reporter Cell Lines for Detection of sIgE

The human high affinity IgE receptor (FcεRI) consists of three different chains, named α , β , and γ . The α chain is responsible for IgE binding, while the other two chains are responsible for receptor stability, signal transduction, and its amplification [7]. Similarly to human mast cells and basophils, RBL cells also possess a homologous endogenous tetrameric FcεRI; however, the rat FcεRI α chain (α_R) does not bind human IgE [8, 9]; RBL cells are therefore not suitable for testing

human sera. To overcome this limitation, several groups have generated stable chimeric RBL transfectants which express either the human FcεRI α (α_H) or all three human chains (see Fig. 1 and Table 1 for details) (reviewed in [21]). In 1992, Gilfillan et al. showed that the α_H chain could functionally substitute the rat homologue chain [16]. The first proof-of-principle studies by Taudou et al. [17], Wilson et al. [22], and Dibbern et al. [12] were published 1 year later. Taudou et al. transfected α_H cDNA into RBL-2H3 and obtained RBL clone T8, which was then used for further studies. The T8 clone only had low α_H expression on the surface (an average of 4100 receptors per cell) despite strong mRNA synthesis, as shown by Northern blot analysis. By using immunoprecipitation experiments, they were able to demonstrate association of the α_H with the rat γ chain (trimeric $\alpha_H(\gamma_R)_2$ configuration). RBL T8 cells were responsive to dust mite antigen Der f 1 after sensitization with human IgE purified from a pool of dust mite allergic patients; however, this involved measurement of calcium influx rather than mediator release [17]. The authors reported high toxicity of some sera when used in high concentrations while attempting to obtain sufficient sensitization of the chimeric rat cells.

Similarly, Wilson and coauthors demonstrated stable α_H expression on transfected RBL-2H3 cells and the ability of human IgE to release mediators (in their case 5-hydroxytryptamine) [22]. Takagi and coauthors used RBL-hEla-2B12, which they had also humanized by transfection and stable expression of α_H , for measurement of IgE levels in human sera [15]. Similarly to Taudou et al. [17], they also found interference of some human sera when used at higher concentrations, however they did not achieve successful stimulation with dust mite extract as cognate antigen.

Dibbern and co-workers assessed the ability of triple human α , β , and γ chain transfectant RBL SX-38 cell lines, created by Wiegand et al. [10], to be sensitized with IgE from

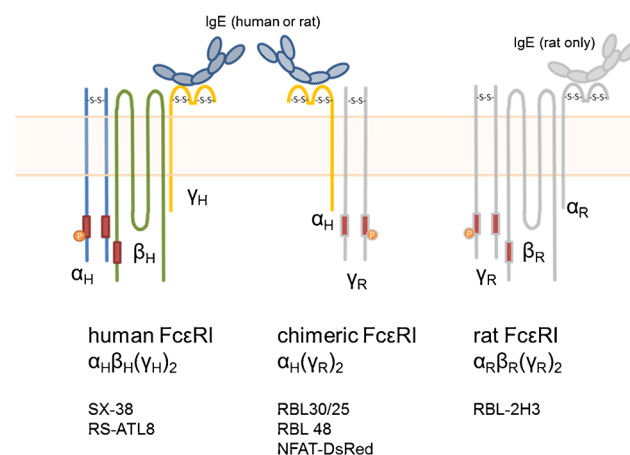


Fig. 1 Schematic diagram summarizing the main different chimeric humanized RBL cell lines described in the literature with respect to human and rat endogenous FcεRI

Table 1 Humanized RBL cell lines

Name of cell line/clone	Transgenes	α_H /cell	Detection	Application tested	Original reference
SX-38	$\alpha\beta\gamma$	100,000 [10]	5-Hydroxytryptamine [10] Beta-hex [11]	sIgE detection [12] Allergen characterization [11]	[10]
RBL-30/25	α	n.d.	Beta-hex (50 % D ₂ O) [13]	Allergen standardization [13]	[13]
RBL-2H3.E5.D12.8	α	n.d.	Beta-hex	sIgE detection	[14]
I5/3/C	α	1800	[³ H] 5-HT	sIgE detection	
H 212IC		3200			
H 7/1/A		2500			
RBL-hE1a-2B12	α	n.d.	Beta-hex	sIgE detection	[15]
RBL 48	α	25,000	Histamine, AA	sIgE detection	[16]
RBL T8	α	4100	Calcium influx	IgE dependent activation	[17]
RBL NFAT DsRed	α	n.d.	DsRed (fluorescence)	sIgE detection	[18, 19]
RS ATL-8	$\alpha\beta\gamma$	n.d.	Luciferase	sIgE detection; allergenicity assessment	[20••]

n.d. not determined

sera of 18 highly peanut-sensitive patients [12]. Spontaneous degranulation due to incubation with sera alone was high (10–25 %), but could be reduced to 5–15 % by cross-absorption of the sera (as 10 % dilution in EMEM) on parental RBL-2H3 cells, which will not bind human IgE, while to some extent removing cytotoxic serum components (such as activated complement). This work showed that with certain highly active sera, humanized RBL cells are able to detect very low amounts of allergen (60 pg/mL for Ara h 1 and 15 pg/mL for Ara h 2) and are very specific. However, a minimum sIgE content of 10 IU/mL (>24.4 ng/mL) of peanut-specific IgE was required, with sera containing >25 IU/mL (>61 ng/mL) resulting in full RBL SX-38 cell activation that was almost undistinguishable from polyclonal activation with an anti-IgE antibody. In agreement with the importance of the sIgE concentration, the authors also found a strong correlation between the amount of sIgE in the sera and their ability to efficiently sensitize the RBL SX-38 cells ($r=0.95$, $p<0.001$), rather than with the total amount of IgE, which barely achieved significance ($r=0.47$, $p=0.05$). Marchand et al., using their own humanized RBL-2H3 cell line (clone E5.D12.8), in contrast did not find a good correlation between sIgE and allergen-induced degranulation, measured as beta-hexosaminidase release [14]. Their work defined a minimum sensitizing dose of 10 ng/mL IgE when stimulated with anti-IgE, as well as a minimum occupancy of 10 % of the receptors with sIgE, for detectable activation. This lower sensitivity was suggested to be due to the less effective signaling activity of trimeric $\alpha_H(\gamma_R)_2$ chimeric IgE receptor in comparison with the endogenous tetrameric $\alpha_R\beta_R(\gamma_R)_2$ configuration.

Lowe et al. used the RBL 48 humanized cell line (the same cell line used by Gilfillan et al. [16]) to estimate the inhibitory biological activity of a humanized monoclonal antibody (rhuMAbE25) on activation of ragweed-specific IgE-sensitized cells upon stimulation with ragweed allergen [23]. Sera from patients with allergies to dust mites, cats, or *Alternaria*

were also successfully used with the RBL 48 cell line. The readout used was measurement of histamine and had an excellent correlation coefficient of 0.93 ($n=59$, $p<0.0001$) with the human basophil histamine assay used in parallel.

Vogel et al. generated a new chimeric RBL cell line designated RBL 30/25, initially through triple transfection with plasmids encoding α_H , β_H , and γ_H chains [24]. However, the stable RBL 30/25 clone expressed only α_H mRNA, despite being fully functional when stimulated with polyclonal anti-IgE. RBL 30/25 gave best responses when sensitized with serum dilutions between 1:20 and 1:40 with an optimal allergen concentration of around 100 ng/mL. The authors also reported the lack of a good correlation between sIgE concentrations and mediator release, and that some sera were toxic at high concentrations. Based on their data, the authors suggested the use of their chimeric cell lines for standardization of allergen extracts (using specific reference sera), rather than as a tool for detection of sIgE in patient sera. More recently, Ladics et al. [25] directly compared the performance of three different humanized chimeric RBL cell lines using sera of peanut-allergic individuals and crude peanut extracts. The three cell lines used were RBL-hE1a-2B12 by Takagi et al. [15], RBL 30/25 by Vogel et al. [24] (both single α_H chain transfectants), and triple transfectant SX-38 by Wiegand et al. [10].

Overall, this work paved the way for the use of humanized RBL cells in allergen standardization as well as pharmacological or immunological inhibitor testing, while at the same time pointing to key issues limiting the usefulness of using chimeric RBL lines for detection of sIgE in serum samples as a diagnostic tool. The main limitation, as pointed out by Ladics, appeared to be the lack of consistency when using sera from different donors; this may be the combined result of low α_H expression levels, a low ratio of sIgE to total IgE in some patients' sera, potentially resulting in insufficient sensitization during incubation with high serum dilutions, and unspecified

toxicity of certain sera. On the positive side, humanized RBL cell lines, when used in combination with some specific sera, showed very high sensitivity when testing for allergens [24] and some potential as a biomarker for clinical severity of symptoms after peanut exposure in sensitized patients [25].

Introduction of Humanized Reporter Cell Lines

Overall however, the disadvantages appeared to override the potential benefits, and as a consequence, no new chimeric cell lines were developed or studied in subsequent years. This only changed with the publication of the EXiLE test in 2010 [20••]. The EXiLE test (for IgE crosslinking (x)-induced luciferase expression) is a further development based on the SX-38 triple chimeric transfectant [10] which was stably transfected with a nuclear factor of activated T cells (NFAT)-responsive luciferase reporter gene construct. The use of such a reporter gene has considerable advantages over traditional biochemical assays conventionally used for detection of RBL activation. RBL-2H3 cells, from which all chimeric cell lines discussed here were created, release histamine upon activation [26]. However, most frequently beta-hexosaminidase has been used as a less cumbersome and expensive surrogate marker of cellular activation and degranulation. Beta-hexosaminidase, also known as N-acetyl-beta-glucosaminidase (EC 3.2.1.52), is a glycosidase which hydrolyses the terminal non-reducing N-acetyl-D-hexosamine residues in N-acetyl-beta-D-hexosaminides. It is a dimeric enzyme which can occur as heterodimer or homodimer (α/α , β/β , or α/β) of two separate subunits which are encoded on two different genes, HEXA and HEXB. The release of beta-hexosaminidase is measured, usually 1 h after stimulation, after addition of a colorimetric or fluorimetric substrate (p-nitrophenyl N-acetyl-glucosaminide or 4-methylumbelliferyl-N-acetyl- β -D-glucosaminide, respectively). Beta-hexosaminidase assays have been used by several groups for experimental determination of sensitization to specific allergens. However as this method is not very sensitive, sensitivity has been frequently increased by using various enhancing agents such as deuterated water (the most common stimulation buffer uses 50 % D₂O) or 5'-(N-ethyl)carboxyamidoadenosine (NECA), an analog of adenosine. The inclusion of D₂O is thought to increase sensitivity by stabilizing microtubules [27] which are involved in the movement of secretory vesicles during exocytosis [28]. In contrast, NECA is thought to increase antigen-dependent degranulation of RBL cells by activating phospholipase C [29]. However, these additions also increase background spontaneous activation, compromising the signal-to-noise ratio [30, 31].

In contrast, the EXiLE system relies on NFAT-dependent expression of the firefly luciferase reporter gene. Hutchinson and McCloskey were able to show that Fc ϵ RI-dependent activation of RBL cells resulted in translocation of NFAT to the nucleus [32]. NFAT translocation is a consequence of the

pronounced calcium release activated channel (CRAC)-dependent secondary influx of Ca²⁺ from the extracellular environment, activating calmodulin-dependent enzymes, leading to sustained calcineurin activation and multiple dephosphorylation of NFAT [33]. The dephosphorylation of 13 of the 14 phosphorylation sites on NFAT leads to unmasking of a previously hidden nuclear localization sequence (NLS) and masking of a nuclear export signal (NES) [34], resulting in nuclear translocation and binding to specific promoters. In the NFAT reporter system (Fig. 2), this results in activation of the reporter gene, hence production of luciferase mRNA, which is translated into luciferase protein after reaching the cytosol and can be measured using appropriate chemiluminescent substrates. This process is very rapid, and luciferase activity already peaks 3 h after activation [20••]. In RBL-2H3 cells, NFAT translocation would result in cytokine and chemokine gene transcription, in combination with other factors such as API [32]. NFAT-dependent mRNA expression of monocyte chemoattractant protein-1 was one of the most prominent changes after antigen stimulation of RBL-2H3 cells, as revealed by oligonucleotide microarray [35].

EXiLE Is a Very Sensitive Technique

One of the main advantages of the RS-ATL8 cells used in the EXiLE system is that because it uses a very sensitive detection technology, in comparison with the traditional biochemical beta-hexosaminidase assay, it does not necessitate any sensitivity-enhancing substances such as the aforementioned NECA or D₂O. This high sensitivity is due to the high quantum yields obtained with the luciferase substrate [36], the robustness of the method, which is unaffected by issues like the presence of quenchers or the temperature, in combination with the low background in unstimulated cells. Another possible reason for increased sensitivity is that less allergen may be needed for optimal activation when measuring NFAT translocation (leading to cytokine and chemokine induction) rather than events linked with degranulation (leading to histamine and beta-hexosaminidase release). It has been shown for human peripheral blood basophils that the optimal concentration needed to obtain maximum release of histamine is 5–10 times higher than that required for optimum cytokine induction (50–100 and 10 ng/mL, respectively) [37, 38].

Altogether these factors allow for the use of higher dilutions of serum (1:100 being recommended as standard dilution in EXiLE [20••]) than those used when assessing cell activation via beta-hexosaminidase. The higher serum dilutions in turn avoid issues of cytotoxicity without compromising too much on sensitivity [39].

Some limitations however remain regarding the concentration of sIgE needed for detection. Wan et al. [40•] used sera

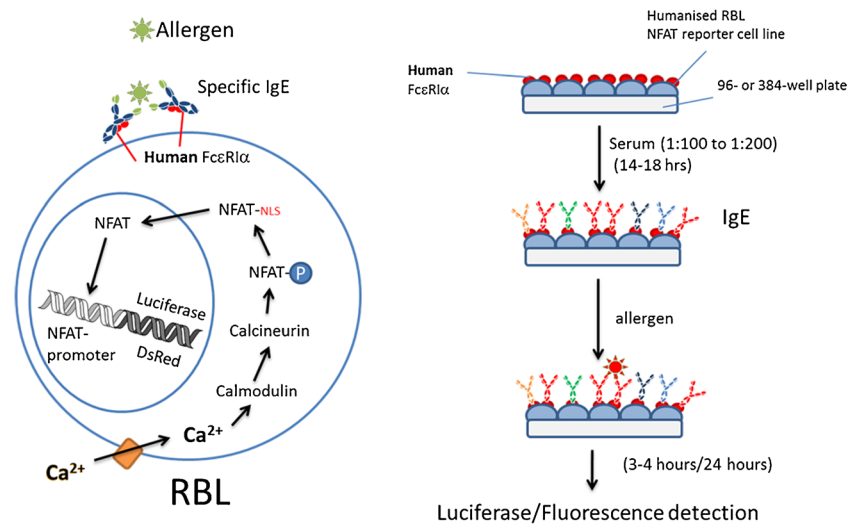


Fig. 2 Schematic diagram illustrating the principle underlying the use of humanized RBL reporter systems for detection of sIgE in patients' sera. Reporter cell lines are incubated overnight with high dilutions of sera to be tested and stimulated the next day with the suspected allergens. In the presence of sIgE, FcεRI crosslinking by the allergens will result in

activating a signal transduction cascade ultimately resulting in the nuclear translocation of NFAT, which activates the reporter gene. This can be measured after 3 h in the case of luciferase or after 16–24 h in the case of DsRed fluorescent protein as reporters

from 11 individuals infected with the blood fluke *Schistosoma mansoni* to sensitize RS-ATL8 cells with subsequent stimulation with SmTAL-1, a prominent allergen from this parasite [41]. Sera were used at 1:100 dilution and contained sIgE levels ranging, as determined by ELISA, from RAST class 2 (moderate IgE, 0.70–3.49 IU/mL or 1.68–8.39 ng/mL) to RAST class 3 (high IgE, 3.50–17.49 IU/mL or 8.40–41.97 ng/mL). Results of activation with SmTAL-1 showed a statistically significant, but relatively weak Spearman rank correlation coefficient of $R_s=0.663$ ($p=0.035$, two-sided). On the positive side, only 1 of the 11 tested sera failed to induce RS-ATL8 activation upon allergen stimulation. This result however also compares very favorably with the previously mentioned minimum sensitizing dose of 10 ng/mL IgE for polyclonal stimulation with anti-IgE (in the presence of 50 % D₂O) postulated by Marchand et al. [14]. The sensitizing sIgE dose used by Wan et al., taking into account the 1:100 dilution, was approximately in the range of 0.04–0.4 ng/mL, without sensitivity-enhancing additives, additional treatments for removal of cytotoxicity, and used antigen-specific (SmTAL-1) rather than polyclonal (anti-IgE) stimulation. This clearly demonstrates the gain in sensitivity obtained when using a luciferase-based reporter system such as the RS-ATL8 instead of non-reporter humanized RBL cells. Our laboratories have used RS-ATL8 in 96-well and 384-well plates with equal success; thus, using this system it is possible to screen for a multitude of recombinant allergens or allergen-containing extracts using only small amounts of patient serum and reagents. This is of particular significance when working with pediatric blood samples.

Use of EXiLE for Elucidation of Complex Patterns of Sensitization

The ready availability and ease of use of EXiLE enables systematic investigations into complex and unexpected patterns of sensitization, which would be very difficult to carry out by traditional methods alone. In two recently published studies [42•, 43], we used RS-ATL8 cells to study an important “outbreak” of systemic allergic manifestations in Japanese individuals which had regularly used a soap product containing a particular acid-hydrolyzed wheat protein (HWP) called Glupearl 19S (Glp19S). More than 2100 regular users of this soap had become sensitized to Glp19S via their skin or mucosa. In these patients, ingestion of wheat-based food, which does not contain HWP, led to various systemic allergic manifestations, including anaphylaxis. These individuals did not react strongly to wheat extracts in SPT, but had strong allergic responses to ingested wheat, particularly in combination with exercise [42•]. The first most obvious conclusion from these observations was the apparent disconnection between the strong clinical manifestations after wheat intake and the relative lack of reactivity in SPT. However, other observations were difficult to explain: Why do the HWP patients develop allergic symptoms when they have eaten wheat that contains no HWP?

It was hypothesized that metabolic changes during digestion and/or absorption of wheat products might result in the generation of new epitopes that are cross-reactive with the epitopes present on HWP. To test this hypothesis, we compared EXiLE responses to control untreated gluten with the

response to gluten digested with pepsin/pancreatin, gluten treated with tissue transglutaminase (tTG), or gluten digested with pepsin/pancreatin first and treated with tTG subsequently. Using these preparations and the EXiLE system, we compared pediatric wheat allergy samples with HWP patient blood samples. The results were very clear: while pediatric wheat allergic samples reacted mainly to untreated gluten, and showed a slightly reduced response to treated gluten, HWP patients did not show any reaction to untreated gluten, but showed a very marked response to tTG-treated gluten. The response to protease plus tTG-treated gluten was weaker, but still clearly measurable. This pointed to the action of tTG as a key step in generating new IgE reactive epitopes (neopeptides) in wheat-containing food leading to allergic manifestations in skin-sensitized HWP patients (Fig. 3). The link between food intake and exercise is still somewhat unclear.

Overall, the picture which emerged from the systematic use of EXiLE in this study is strongly reminiscent of what is found in celiac disease. In celiac disease, wheat gliadin peptides are deamidated by tTG, and presented on HLA DQ2/DQ8 to activate self-reacting T cells, causing increased permeability of wheat peptides and disease propagation [44]. Patients must avoid wheat ingestion throughout their lives. Considering that industrially generated acid-hydrolyzed Glupearl 19S is highly deamidated, it was suggested that the HWP patients were sensitized to these deamidated structure through the skin, and that once produced, the IgE would recognize the deamidated gluten peptides absorbed in the small intestine of these patients.

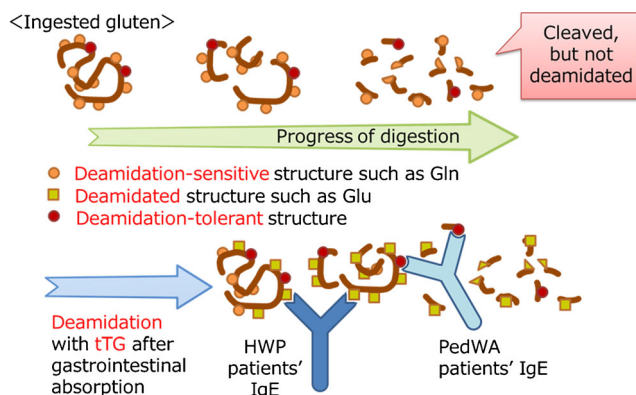


Fig. 3 Suggested changes in the molecular structure of gluten after ingestion, due to the action of digestive proteases (pepsin, pancreatin) and tissue transglutaminase (tTG) leading to formation of IgE-reactive neopeptides. *Gln* glutamine, *Glu* glutamate, *HWP* acid-hydrolyzed wheat protein, *PedWA* pediatric wheat allergy. Glutamine-rich gliadin is degraded in the stomach through the combined action of gastric acid and pepsin, but initially not deamidated. Tissue transglutaminase in the small intestine results in deamidation and generation of neopeptides which are recognized by IgE in HWP patients, but not in pediatric wheat allergic patients

Combining Allergen Arrays With Basophils: a New Powerful Approach for Clinically Relevant Detection of Sensitization?

The advent of protein arrays has enabled simultaneous screening of IgE binding to a large number of different allergens using only a small amount of serum, a process particularly advantageous to patients with suspected polysensitization [45]. These tests have been performed in “traditional” array format using complex extracts or purified/recombinant allergens (component-resolved or molecular-based diagnosis) and antibody detection [45–50] in humans and very recently also applied to the study of equine sensitization to insect bites [51]. Thus far, this approach does not fully mirror the biological (cellular) dimension of allergy. Are allergen arrays amenable to a biological readout of cellular activation?

We have previously shown as proof-of-principle that allergen arrays can be combined with purified peripheral blood basophils providing a cellular readout, rather than merely reporting IgE binding to solid phase-bound allergens [52]. In these experiments, allergens arrayed on nitrocellulose-coated slides were incubated with human basophils. These had to be purified to near homogeneity from a healthy donor’s peripheral blood, using a three-step protocol [53], and the IgE stripped by lactic acid incubation before resensitization with the patient’s serum. Although faster protocols for basophils are now available [54], this approach is still limited by the cost and the difficult logistics of purifying sufficient amounts of basophils from peripheral blood and analyzing the cellular response within a few hours after purification. It is clear how such a complex setup, despite the potential to give useful and clinically relevant information, would remain confined to a research laboratory without ever entering clinical practice. This consideration drove us to assess the use of humanized reporter cell lines in combination with allergen arrays [18]. The RS-ATL8 reporter used in EXiLE is not suitable for this format because luciferase activity measurement requires cell lysis, which would disconnect signals from the allergen spots in a solid-phase array. Therefore, alternative reporter systems based on intracellular fluorescence, such as the DsRed and GFP reporters developed in our lab, had to be developed [18]. One advantage of such reporter systems is that once incubated with the test serum there is no necessity to use any additional reagents or substrates. Therefore, this system can be used in high-throughput format using 96- or 384-well plates or a solid surface with easy and inexpensive protocols [19].

Thus, it now appears feasible to combine high-throughput screening of allergens using only small amounts of serum with a readout reporting cellular activation, rather than sIgE binding. However, it is still necessary to demonstrate in head-to-head analyses that the array method in combination with humanized RBL reporter systems results in clinically more relevant diagnostic results.

Taken together, a new generation of humanized RBL reporter systems has successfully overcome the limitations which in the past have restricted the use of such systems to research units in universities and hospitals. The luciferase- and fluorescence-based new systems potentially represent a powerful addition to the technologies that can enable the long sought-after development of economical, clinically relevant, and robust tools for the diagnosis of allergic sensitization.

Conclusion

Humanized RBL reporter systems, in their latest incarnation as fluorescent or luciferase reporter systems, have overcome many of the limitations of their older non-reporter counterparts. Their high sensitivity and robustness make them attractive tools for standardization of allergens or assessment of allergic sensitization in humans. A generation of humanized reporters has been created, which allows for the first time the combination of biological relevance of a cellular readout with the numerical power of allergen arrays in allergy testing.

Whether such a technology is able to significantly improve the status quo of diagnostic technologies remains to be demonstrated using well-characterized clinical samples in large study cohorts.

Compliance with Ethics Guidelines

Conflict of Interest Drs. Falcone, Alcocer, Okamoto-Uchida, and Nakamura declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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