

The IgE-microarray testing in atopic dermatitis: a suitable modern tool for the immunological and clinical phenotyping of the disease

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Purpose of review

To define the role of IgE-microarray testing in atopic dermatitis where the multifunctional environmental factors implicated in the pathogenesis of the disease deserve a complex and exhaustive approach for the finest definition of relevant allergenic triggers, if any.

Recent findings

Allergenic factors involved in atopic dermatitis have been reported in inducing atopic dermatitis. Environmental triggers, such as aeroallergens, food allergens, bacterial, and fungal antigen, and the presence of IgE to self-antigens have been described as well. The usefulness of the microarray testing has been preliminary described.

Summary

As far as the number of potential triggers or worsening factors in atopic dermatitis is quite broad, we foresee a crucial role for the IgE microarray testing. The microarray testing is helpful in defining all additional reactivity the atopic dermatitis patient could have acquired in her/his allergic life, mostly related to inhalant allergens. Nevertheless, interpretation needs skills and thus this new technology should rather be reserved for the allergologist as it may lead to false conclusions if broadly used in general medicine. IgE microarray testing gives trustable results to define the nonallergic atopic dermatitis form because of the broad and comprehensive negative IgE testing.

Keywords

allergenic molecules, atopic dermatitis, diagnosis, IgE, microarray

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Introduction

The skin plays an essential role in the protection from a variety of potential pathogens coming from the external environment. It represents more than a simple passive barrier: the mechanical attributes are strengthened by the resident immune system of the skin, which is essential in the preservation of the human body homeostasis. The relevance of the skin resident immune response is made more and more evident observing the involvement of the skin in allergic diseases, in a number of genetic and acquired immunodeficiency disorders, in cutaneous tumors such as Mycosis Fungoides and Sézary Syndrome, and in patients receiving immunosuppressive treatments after organ transplantation.

Approximately one-third of the population worldwide is affected by allergic diseases. There are several reports suggesting the presence of genetic and epigenetic factors involved in this phenomenon. However, the precise recognition of genes implicated is not straightforward, because of the composite interactions of a range of molecules in different phases of the allergic reaction, a

complex interplaying of environmental factors, such as protein allergens, irritants, and toxic compounds (e.g., endotoxins), and fungal, viral, or bacterial proteins, and various, often associated, clinical phenotypes (i.e. asthma, allergic rhinitis, atopic dermatitis, or allergic conjunctivitis).

In atopic dermatitis, affecting up to 20% of children and between 0.2 and 8.8% of adults, the partial loss of the cutaneous barrier function, due to low ceramide levels and reduced filaggrin function, facilitates the trans-epidermal water loss [1,2] and the penetration of environmental compounds, including allergens or pathogen-derived toxins, thus facilitating a specific, IgE-driven, allergic skin inflammation.

Because of the huge number of factors possibly involved, there is a need of innovative investigative tools allowing the analysis at the same time of a great number of sensitizers and elicitors of IgE reactivity, or to exclude them all at the end. The proof of concept for detecting allergen specific IgE using microarray technology was given almost 10 years ago by Hiller *et al.* [3], and its use in

the diagnostic routine, and at the same time, as a high throughput investigative tool has been recently reported in a milestone study by Scala *et al.* [4**].

The scope of the present review is to explain the role and added value represented by the IgE-microarray testing in the study and management of atopic dermatitis, taking into account the need for a detailed IgE profiling using allergens/antigens of many kinds, the co-morbidity with other allergic diseases, and the similarity of the latter with atopic dermatitis. As far as very few or no studies specifically addressing the use of microarray IgE testing in atopic dermatitis have been published, we also report some original and preliminary epidemiological data on the topic from the data repository of our Center.

Allergenic triggers in atopic dermatitis: from the far outside to the inseparable inside

Unless a clear cut separation of the environment outside of the human body from inside of it can hardly immunologically be achieved, three main groups of the allergen way of exposure in atopic dermatitis will be described, and relationship among them, if any, reported. A comprehensive list of allergens involved in atopic dermatitis can be retrieved using the RefArray tool available on the Allergome platform (www.allergome.org/script/refArray.php) using a text search with 'eczema'. A list of selected studies dealing with 'diagnosis' or 'epidemiology' can easily be obtained for each listed allergen.

Environmental sensitizers/triggers

Environmental allergens reported to be involved in the pathogenesis of atopic dermatitis include both food allergens as well as aeroallergens [5*].

Aero-dispersed allergens

Previous studies showed that aeroallergens such as house dust mite, pollen, or fungal spores might trigger atopic dermatitis [6,7]. Among children with atopic dermatitis, there is a correlation between the severity of atopic dermatitis and the degree of sensitization to aeroallergens, probably enhanced by damage of the skin barrier function [8]. Similarly aeroallergen avoidance, mostly house dust mites, can result in marked improvement of skin lesions [9]. Michel *et al.* [10] demonstrated that cockroach allergens can induce a positive skin test reactivity and cockroach specific IgE in patients with atopic dermatitis. Fernandes *et al.* [11] reported mite tropomyosin acting as one of the major house dust aeroallergens in patients affected by atopic dermatitis and allergy respiratory disorders. Japanese cedar pollen has been described as a cause of seasonal worsening of atopic dermatitis in patients tested IgE positive to a pollen extract with a known amount of Cry j 1 [12].

Key points

- The microarray technology for routine IgE testing is available and mature.
- Atopic dermatitis patient deserve a broad IgE testing.
- Atopic dermatitis and related allergic diseases should be studied at once.
- Allergens involved include proteins from the environment and self-antigen.
- The absence of specific IgE can be defined just by a large number of negative results.

During the last few years at the Center for Molecular Allergology (CAM, IDI-IRCCS, Rome, Italy), the specific IgE reactivity using a molecule-based microarray-based system has been measured in 1165 patients (61.29% in the pediatric age) affected by atopic dermatitis, as diagnosed following established international criteria. Top ranked IgE positive molecules were the timothy grass allergen Phl p 1 (46% of patients) followed by the house dust mite allergens Der p 2 and Der f 2 (42.5 and 40.9%, respectively), and by the cypress pollen allergen Cup a 1 (39.3%) (CAM, IDI-IRCCS; data on file). This preliminary reported survey underlines the need for detecting IgE sensitization to environmental allergens, using the above maker molecules or the broadly co-recognized ones (e.g. profilins) for single patient IgE profiling. Foods having IgE cross-reactivity with pollen may also be clinically important for adult and children with atopic dermatitis [13]. PR-10 present in plant-derived food may lead to an exacerbation of eczema in a subset of adult patients with atopic dermatitis [14]. A subset of adult atopic dermatitis patients had late worsening of atopic dermatitis lesions after oral challenge with birch pollen-related food, and the vast majority of atopic dermatitis patients were not conscious of the possible involvement of these foods in their skin disease [15]. A birch pollen-specific T-cell response can also be found in lesional skin of these patients [14].

Using the Immuno Solid phase Allergen Chip (ISAC microarray) (Phadia Multiplexing Diagnostics, PMD, Austria), our Italian cohort of patients with atopic dermatitis showed an IgE reactivity to Lipid Transfer Proteins in 18.9% of the patients, whereas PR-10 and profilin IgE sensitization was recorded in 14.7 and 7.5%, respectively (CAM, IDI-IRCCS; data on file). Such data not only confirm the relevance of plant-derived allergen in atopic dermatitis, but also show a higher prevalence of such sensitizations when comparing the same allergen IgE reactivity with a larger allergic population affected by any allergic diseases [4**].

Food allergens

Three distinct patterns of cutaneous adverse reactions to food can be observed in patients with atopic dermatitis:

an immediate reactions (urticaria, angio-oedema), usually occurring few minutes after double-blind placebo-controlled food challenges (DBPCFC), without exacerbation of atopic dermatitis; itching immediate reaction followed by exacerbation of atopic dermatitis, and late reactions with a worsening of atopic dermatitis, even several hours after the challenge (6–48 h) [14]. The patients' history of food-related eczema does not have a high diagnostic specificity [16]. An association could exist between severity of atopic dermatitis and high total IgE levels [17], although some evidence suggests that up to two-thirds of patients with atopic dermatitis are not atopic [18]. Around one-third of the patients with moderate to severe atopic dermatitis have a DBPCFC proven food allergy [19]. Anyhow, attention should be undertaken while evaluating the atopic dermatitis patient about having food allergy. It is important to keep in mind that a subset of children with atopic dermatitis have food allergy, but the largest percentage has clinically irrelevant food sensitization. From an allergy diagnosis based on allergenic molecules, the clinical relevance of food sensitization can be easily clarified and in case rule out its clinical relevance by detecting for instance IgE to CCD [20], or to profilin [21]. Both these allergenic determinants are rarely, if ever, responsible of adverse reaction unless they are present in all plant derived food sources studied so far. Such testing could avoid dietary avoidance for the prevention of a nonexistent disease [22], that could be nutritionally harmful, and impose unnecessary anxiety on children and caregivers [23]. Conversely, oral food challenge is recommended to confirm clinical relevance of sensitization and to ensure the need for food avoidance diet. Although 90% of food allergy in children with atopic dermatitis is associated with hen's egg, milk, wheat, soy, peanut, nuts, and fish IgE reactivity [24], an allergenic molecule-based profiling has never been extensively performed so far.

Cow's milk allergy ranges from mild to severe symptoms engaging the skin, the respiratory and/or gastrointestinal tracts up to life threatening systemic reactions. Little is known about the IgE recognition profile of patients, and if the recognition patterns could differ from patient to patient, and if the differences are reliably related to symptoms. Werfel *et al.* [25] showed a kappa-casein-specific T-cell-mediated immune response in many patients with a history of milk-related exacerbation of atopic dermatitis and about 3% of children suffering atopic dermatitis had symptoms on ingestion of not well cooked beef due to bovine serum albumin (Bos d 6) and bovine gamma globulins (Bos d 7) reactivity [26]. Recently, Hochwallner *et al.* [27••] employed a microarray assay containing highly purified recombinant cow's milk allergens and natural milk allergen preparations for the analysis of the IgE reactivity profiles in cow's milk allergic patients. The microarray included 27 milk prep-

arations of purified natural milk proteins (α -casein; β -casein; k-casein; α -lactalbumin; human α lactalbumin; β -lactoglobulin variant A and B; BSA; sheep serum albumin; human serum albumin; lactoferrin), casein fractions from different mammals (goat, cow, and sheep casein fraction), milk extracts from cow, sheep, goat, mare, human milk, and recombinant cow's milk α -lactalbumin, β -lactoglobulin variant A and B, and k-casein and three fragments spanning the whole sequence of bovine serum albumin. Eighteen patients suffering only from atopic dermatitis recognized a low number of cow's milk allergens spotted on the microarray and showed weak or no reaction in the basophil degranulation test, whereas all patients with severe systemic reactions and the majority of patients with gastrointestinal symptoms showed basophil degranulation and recognized a large number of different cow's milk proteins.

IgE antibodies do recognize specific food allergen epitopes. Patients able to recognize sequential epitopes seem to clinically react even when foods are extensively cooked [28]. The same patients show more persistent allergy [29]. Moreover, the diversity of epitope recognition, as detected by a peptide-based microarray, has been associated with the severity of an allergic reaction. Overall data exist about different epitope IgE recognition on most important cow's milk allergens and their role in skin disease in cow's milk allergic children [29–35].

Hen's egg sensitization is considered a marker of atopy as 70% of children suffering from atopic dermatitis have egg white specific IgE. Along with a positive family history of atopy, they develop recurring wheezing by the age of 5 years [36,37], thus representing a risk factor and reinforcing the need for IgE testing. We recently reported about 25 children with suspected egg allergy detecting IgE to egg allergens available on the ISAC 103 microarray (PMD, Austria), namely Gal d 1 (ovomucoid), Gal d 2 (ovalbumin), Gal d 3 (conalbumin), and Gal d 5 (livetin). Selected patients reacted to double-blind placebo controlled food challenge; sixteen of them showed also a flare of their atopic dermatitis. Different IgE positive results were obtained depending on egg white or yolk recognition. Beside egg allergens, 23 children showed sensitizations to other foods and inhalants confirming that multiple specific IgE evaluation by means of ISAC improves the diagnostic approach in hen's egg allergic children affected by atopic dermatitis [38•].

Even if wheat-allergic atopic dermatitis patients have IgE antibodies against gliadin [39], the responsible allergens in wheat food allergy are not well defined yet, and problems exist in screening patients who would benefit from elimination diets. A wheat flour allergen microarray could improve diagnosis as has been shown in baker's asthma [40].

Patients never exposed to peanut by ingestion can acquire sensitization as a result of application of peanut oil containing cream on damaged atopic dermatitis skin [41]. Whether the cutaneous or the ingestion routes of exposure cause specific allergen IgE recognition profiles in atopic dermatitis patients has never been reported. Young children with atopic dermatitis sensitized to hazelnut can display IgE reactivity to Cor a 9, a potentially dangerous hazelnut component as it has been shown analyzing their sera with the allergen microarray immunoassay ISAC [42^{••}]. We found a prevalence of 5–7% of reactivity to Cor a 9 and an overall prevalence of 20.1% of seed protein reactivity in our atopic dermatitis population (CAM, IDI-IRCCS; data on file). As for inhalant allergens such percentages are much higher than those recorded in the general allergic population.

Several other food allergen sources, including fish, crustacean, soy, have been reported as positive in atopic dermatitis affected patients [5[•],43]. Unless allergenic molecules have been identified for those sources, no data are available on IgE recognition profile in atopic dermatitis patients. From our atopic dermatitis cohort, the fish parvalbumin available on ISAC was recorded IgE positive in 5.9% of the patients, whereas shrimp tropomyosin and genuine soy allergens were IgE positive in 12.1 and 6.8% of the atopic dermatitis patients, respectively.

Skin surface sensitizers/triggers: bacterial and fungal allergens

The skin of individuals suffering from atopic dermatitis clearly show increased vulnerability to cutaneous bacterial or fungal colonization [44]. The predominant skin infection in atopic dermatitis is caused by *Staphylococcus aureus*. The presence of specific IgE antibodies to *Staphylococcus* exotoxins in patients with atopic dermatitis can be considered as an important suggestion for the role of microorganisms as allergic trigger in atopic dermatitis [45]. The presence of specific IgE to *Staphylococcus* exotoxins should be at least considered a marker of exposition to these antigens, thus addressing the need for specific antibacterial intervention. Furthermore, these exotoxins may play a central role not only as ‘allergens’ by specific activation of FcεRI⁺ cells, but also as ‘super-antigens’ through non-Major Histocompatibility Complex restricted activation of large numbers of T cells bearing the appropriate TCRVβ family. *S. aureus* strains generate a number of exotoxins with super-antigenic capacity, such as *Staphylococcus* exotoxins-A, *Staphylococcus* exotoxins-B, *Staphylococcus* exotoxins-C, *Staphylococcus* exotoxins-D, *Staphylococcus* exotoxins-E, and toxic shock toxin 1 (TSST-1) [46–48]. All these super-antigens can amplify the expression of skin-homing receptors (e.g. cutaneous lymphocyte-associated antigen) on T cells and might induce the appearance of skewed

T-cell clones [46,47]. For example, TSST-1 can stimulate selectively TCR-Vβ2 bearing cells, *Staphylococcal* enterotoxin C may bind both Vβ5.1 and Vβ13.1, *Staphylococcal* enterotoxin B and *Staphylococcal* enterotoxin C can excite Vβ17 and *Staphylococcal* enterotoxin E can recognize Vβ5.1 [49–51]. A repeated super-antigen stimulation of the TCR usually causes an activation-induced cell death [52], but the well known resistance to apoptosis of Th2 cells [53] could explain why a repetitive super-antigen stimulation might eventually result in T cell oligoclonality in atopic dermatitis [54]. As a result, treatment of *S. aureus* skin infections with specific antibiotics significantly reduces clinical severity of the disease.

Thus, there are three very good reasons to include detection of IgE to *Staphylococcus* exotoxins in a microarray-based investigation in atopic dermatitis patients.

A restricted subset of patients exhibit IgE reactivity to *Escherichia coli* antigens. Specific IgE recognition was mainly directed to protein bands in the 30–66 kDa molecular weight range [55^{••}]. Patients with moderate to severe atopic dermatitis seem to have more frequently IgE to bacterial antigens than patients with mild symptoms [55^{••}].

Fungi represent common and important airborne allergens, and fungal sensitization to *Candida albicans*, *Alternaria alternata*, *Trichophyton rubrum*, *Penicillium chrysogenum*, *Aspergillus fumigatus*, and *Malassezia furfur* is often observed in atopic dermatitis patients [56,57[•]]. Furthermore, IgE sensitization to *Malassezia* allergens is recorded in atopic dermatitis and not in patients with allergic rhinitis or asthma without atopic dermatitis [58,59]. Recent reports illustrated the presence in atopic dermatitis of a specific IgE recognition of manganese superoxide dismutase (MnSOD), a protein possibly involved in IgE-mediated autoreactivity [60]. The hypothesis of a molecular mimicry followed by cross-reactivity was raised by Schmid-Grendelmeier *et al.* [61], as the result of primary sensitization to MnSOD belonging to *Malassezia sympodialis*. We found 4.8% of prevalence of Asp f 6 IgE recognition, the MnSOD from *A. fumigatus*, whereas Alt a 1, a genuine marker of inhalant allergy to *Alternaria*, represented the most frequent mould sensitization (13%) in our cohort of atopic dermatitis patients (CAM, IDI-IRCCS; data on file).

Self-sensitizers/triggers: mimickers of environmental allergens (or the reverse?)

IgE-mediated reactivity against self-proteins with structural similarity to exogenous allergens has been hypothesized as a further potential mechanism involved in the pathogenesis of atopic dermatitis [62]. In addition to

exogenous allergens, a specific IgE response is directed against a number of human proteins located in a variety of cell types and tissues [62]. Furthermore, a close relationship has been observed between IgE auto-reactivity and atopic dermatitis severity [63]. An IgE response decline was recorded after successful cyclosporin A treatment [64], thus suggesting a role of IgE auto-reactivity at least as a marker of tissue damage [62].

Five IgE-reactive auto-allergens, as officially reported in www.allergen.org as Hom s 1 to Hom s 5, have been identified using sera from patients with severe atopic dermatitis on a human cDNA library. Hom s 1 is SART-1, a Leucine zipper DNA binding protein, identical to a formerly identified tumor antigen [65]; Hom s 2 is the α chain of the nascent polypeptide associated complex, a conserved protein involved in ribosomal protein biosynthesis [66,67]; Hom s 3 is the B cell lymphoma 7B protein (BCL7B) oncogene [63]; Hom s 4 is a calcium-binding protein [63,68]; Hom s 5 is a cytokeratin type II, involved in the formation of intermediate filaments [63].

Including a panel of auto-allergens for detecting auto-IgE using a microarray-based investigation in atopic dermatitis would be of great relevance as their presence could differently address decision making in atopic dermatitis overall therapeutic approach.

Absence of specific IgE-mediated triggers of atopic dermatitis

The role of the overall spectrum of allergens, potentially involved in the primary and secondary allergic response in atopic dermatitis patients, can be ruled out just by IgE testing. It seems to us that there is no other way to go than using the microarray approach for that. The so-called 'intrinsic atopic dermatitis', if it really exists, would be clearly defined by recording a huge number of negative results. Such a goal cannot be achieved by any other means than the microarray testing for technical (i.e. too many singleplex IgE determinations are very expensive, and exhaustive skin testing cannot be performed on the atopic dermatitis affected skin), safety, and ethical reasons (e.g. self-antigen cannot be tested using the skin test).

Application of the herein reported approach to skin and nonskin allergic diseases other than atopic dermatitis

Atopic dermatitis is very often a 'not stand alone' disease. It either precedes or is accompanied by other allergic diseases. This makes the above-described approach for atopic dermatitis suitable for other allergic skin diseases, mainly urticaria, and nonskin diseases, as the respiratory ones (i.e. asthma and rhinitis).

As chronic urticaria is often complicated by atopic dermatitis and some patients with atopic dermatitis have immediate-type reactions in response to intradermal injections of autologous sweat [69], and their basophils induced histamine release in response to the sweat samples [70], it was supposed that patients with chronic urticaria may be sensitive to the same antigen in sweat as patients with atopic dermatitis [71]. Chronic urticaria is associated with thyroid autoimmunity in most cases [72]. Very recently, IgE against the thyroid peroxidase antigen has been reported to be present in chronic urticaria patients [73]. Thus, many etiological aspects could be shared by atopic dermatitis and chronic urticaria, and the co-morbidity for the two diseases leads to consideration of the microarray testing as a valuable approach.

Atopic dermatitis and bronchial asthma are frequently associated features in allergic patients. Similarly to what is observed in atopic dermatitis, a non-IgE-mediated kind of asthma is well described in patients with negative skin tests, a normal amount of total IgE, and no specific IgE to common environmental or food allergens [74]. Similarly to what is observed in nonallergic atopic dermatitis, the symptom onset is recorded later in life [75]. The use of a multiplex approach in the diagnosis of nonatopic bronchial asthma, as in intrinsic atopic dermatitis, might help in clinical practice in order to reliably rule out the presence of IgE sensitization and address treatment to drugs rather than to environmental control exposure.

Conclusion

Allergen-based microarray is a tool for the detection of IgE-related sensitization to a large panel of allergens and antigens in atopic dermatitis, in all associated allergic disorders, and when atopic dermatitis is present in difficult to diagnose diseases. It represents nowadays the finest and the most comprehensive evaluation by using single measurements and minute amounts of serum in patients of all ages. It is noteworthy that such broad testing cannot be achieved by any other previous diagnostic tool, including skin prick test and IgE detection by laboratory singleplexing systems. The release of a more comprehensive microarray including all potential allergens/antigens discussed in the present review should be encouraged. Immunological profiling of every single patient, even in the routine diagnostic workup, could be then easily achieved. Nevertheless, the interpretation of the microarray results requires skills. Thus, this new technology should be in the hands of molecular allergologists to avoid wrong conclusions if broadly used by general practitioners.

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Conflicts of interest

The authors have no conflicts of interest to declare.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 501).

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