



Molecular profile clustering of IgE responses and potential implications for specific immunotherapy

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Purpose of review

To summarize recent data on molecular profiles of IgE sensitization in allergic patients and discuss how they can influence our understanding of allergen specific immunotherapy.

Recent findings

In childhood, Immunoglobulin E (IgE) sensitization to grass pollen starts preclinically as a weak, mono or oligomolecular response and evolves rapidly to become strong, polymolecular and associated with clinical manifestations. This immunological phenomenon has been defined 'molecular spreading' and it makes the IgE sensitization profiles to complex allergenic sources highly heterogeneous in the population.

Summary

The recent findings raise new questions: do different molecular sensitization profiles (e.g. to grass pollen) underlie different clinical responses to allergen-specific immunotherapy? Should the allergen-specific immunological intervention be anticipated at earlier stages of the IgE sensitization process? Should the regulatory rules for molecularly designed allergen-specific immunotherapy preparations consider the extreme heterogeneity of sensitization profiles in populations?

Keywords

grass pollen, IgE, molecular allergy, prevention, specific immunotherapy

INTRODUCTION

Allergen-specific immunotherapy (SIT) is considered the only treatment of allergic rhinitis and asthma with potential long-lasting effects, which may continue after treatment discontinuation [1] and contribute to the prevention of disease progression [2]. SIT has been traditionally based on the administration of extracts of allergenic sources, which are supposed to contain all the major allergenic proteins. However, the exact composition of subcutaneous immunotherapy [3] and sublingual immunotherapy (SLIT) [4] preparations cannot be defined with extreme precision. Moreover, extracts of the same allergenic sources produced by different companies may differ in their molecular composition [5]. To optimize SIT standardization and efficacy, Valenta *et al.* [6,7] proposed the concept of 'Component Resolved Therapy' (CRT): accordingly, a SIT preparation should be characterized and standardized at a molecular level, and its composition should match the patient's molecular profile of Immunoglobulin E (IgE) sensitization.

This concept of 'tailored' CRT is extremely interesting from a theoretical point of view and has stimulated epidemiological and clinical research

targeted at characterizing at molecular level the prevalent IgE responses of patients with respiratory allergies. Accordingly, many studies in the last two decades have examined the frequency distribution of the IgE responses against individual allergenic proteins of the most important allergenic sources. The case of allergenic proteins of Timothy grass, one of the most relevant grass pollen species, is very useful to describe the complexity of IgE sensitization against a single allergenic source. These studies have shown that the frequency of IgE reactivity to the eight major allergenic proteins of Timothy grass is relatively similar in patients' population from different European countries. The major characteristics of these eight molecules are reported in Fig. 1 [8,9]. Phl p 1 is the most frequently recognized protein,

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KEY POINTS

- Allergic patients reacting to the same allergenic source (e.g. grass pollen) can be highly different in their molecular profile of IgE sensitization.
- The IgE response starts simple and weak in childhood and in most patients becomes molecularly more complex ('*molecular spreading*') and quantitatively stronger with time.
- The degree of molecular complexity and of intensity of IgE sensitization in the individual patient may affect his/her clinical response to allergen-specific immunotherapy.
- Allergen-specific immunological intervention may be more effective at the earliest, even preclinical stages of the IgE sensitization process.

followed by Phl p 5, Phl p 4, Phl p 6, and Phl p 2, then by Phl p 11, Phl p 12, and Phl p 7. This hierarchy probably reflects the intrinsic allergenic potential of each of these proteins. Similarly, other complex inhaled allergenic sources also contain several distinct allergenic proteins and the frequency of IgE sensitization to these proteins has been extensively examined in many populations (see example of mites). However, the description of the frequency of sensitization to individual proteins in a population of patients does not answer the question of

how to design a set of allergenic preparations to be used for tailored CRT in that same population. On the contrary, all the above mentioned cross-sectional studies do not answer the question of whether the individual IgE response to a given allergenic source is stable or evolves over time. Both pieces of information are essential in understanding whether and how allergen-specific immunotherapy can be upgraded to face the challenge of the 'molecular' era of Allergy.

The target of this review is to summarize how the most recent studies have just begun to explore this unknown territory by using not only cross-sectional analysis of frequencies based on traditional singleplex assays, but also combinatorial analysis, microarray technology, and sera-banks from birth cohort studies.

IGE SENSITIZATION PROFILES

When examined with an allergenic extract of Timothy grass, grass-pollen allergic patients all look the same from a qualitative point of view: that is, they are all 'positive' (although with different degree of positivity). By moving from extract to molecular analysis, the allergist experiences an upgrade in analytical power similar to that experienced by the pathologist from naked eye to microscope, or the radiologist from radiograms to magnetic resonance. The population's 'apparent' homogeneity is substituted by an enormous heterogeneity. A series

	Structure*	Molecular weight (kD)*	Biological function^	IgE positivity among Europeans sensitized to grass pollen (%) ^
Phl p 1		33–36	Expansin	68–96
Phl p 4		55–60	Berberine bridge enzyme	70–88
Phl p 5		29–38	Ribonuclease	50–100
Phl p 2		11–12	Unknown	28–68
Phl p 6		13	Unknown	44–68
Phl p 11		20	Trypsin inhibitor	35–53
Phl p 12		14	Profilin	10–15.2
Phl p 7		6	Calcium binding protein	5–8

*www.proteinmodelportal.org (June 2012)
 ^Andersson K, Lidholm J. IAAI 2003
 ^www.allergome.com (June 2012)

FIGURE 1. Characteristics of the eight molecules of *Phleum pratense* most frequently used in molecular diagnosis of IgE sensitization.

of 39 different IgE sensitization profiles could be observed in a cross-sectional study [10¹¹] of only 177 Italian children allergic to grass pollen (Fig. 2). The same population of ‘grass pollen allergic children’ included patients sensitized to only one molecule

(in most cases Phl p 1) and patients sensitized to all the eight allergenic molecules examined and 36 additional profiles included between these extremes. Given the complexity of describing each combination of presence or absence of antibodies, the study proposed a so-called ‘Allergen Profile Codification System’ (APCS), based on the decimal conversion of the binary number obtained assigning 1 or 0 to IgE positivity or negativity, respectively, for each of the eight Phl p molecules, once sorted by growing WHO nomenclature. As an example, the decimal code ‘162’, equivalent to 10100010, identifies the IgE sensitization profile of a child with IgE against Phl p 1, Phl p 4 and Phl p 11 and with no IgE to the remaining five molecules. This codification system may help comparing studies of different groups and populations. The study [10¹¹] also demonstrated that a tailored CRT approach should be very flexible. Present regulations, however, require that each preparation should be individually tested. Given the extremely complex and costly regulatory procedures, no company would ever be able to introduce as many preparations as needed at population level to fully implement patient tailored CRT. Tailored CRT will be applicable in routine clinical practice only when these financial and regulatory obstacles are removed [11,12¹²].

DO IGE SENSITIZATION PROFILES MATTER CLINICALLY?

Measurement of IgE antibodies to food allergenic molecules is useful in the diagnosis of food allergy. For example, this has been well demonstrated in the investigation of reactions to raw and roasted peanut: different sensitization profiles to peanuts are associated with different degrees of severity of clinical reactions [13¹³]. Consequently, by testing for Ara h 2 patients allergic to peanut, the need for peanut challenges could be reduced by at least 50% [14¹⁴]. However, among patients sensitized to peanut, those with IgE to Ara h 2 and to Ara h 1 or 3 more often reported peanut symptoms (particularly respiratory symptoms) than children with IgE only to Ara h 2 [15]. IgE analysis to peanut allergen components may then be used to distinguish between peanut-sensitized individuals at risk of severe symptoms and those likely to have milder or no symptoms to peanut if sensitized to pollen allergens and their peanut homologue molecules [15]. Similarly, IgE sensitization to Ovomucoid is a marker of stronger severity of Egg allergy and reactivity to boiled egg [16¹⁶].

Measurement of IgE antibodies to airborne allergenic molecules is also useful in the diagnosis of respiratory allergies. In particular, the identification

APCS code	pt 8 0.35	rPhl p 1	rPhl p 2	rPhl p 4	rPhl p 5	rPhl p 6	rPhl p 7	rPhl p 11	rPhl p 12	n pos. mol.	n	%	cum. %
128	10000000	●								1	36	20.8	21
248	11111000	●	●	●	●	●				5	21	12.1	33
160	10100000	●		●						2	10	5.8	39
184	10111000	●		●	●	●				4	8	4.6	43
186	10111010	●		●	●	●		●		5	8	4.6	48
251	11111011	●	●	●	●	●		●	●	7	8	4.6	53
192	11000000	●	●							2	7	4.0	57
216	11011000	●	●		●	●				4	7	4.0	61
249	11111001	●	●	●	●	●			●	6	7	4.0	65
250	11111010	●	●	●	●	●		●		6	7	4.0	69
32	100000			●						1	5	2.9	72
224	11100000	●	●	●						3	5	2.9	75
152	10011000	●			●	●				3	4	2.3	77
185	10111001	●		●	●	●			●	5	4	2.3	79
208	11010000	●	●		●					3	3	1.7	81
218	11011010	●	●		●	●		●		5	3	1.7	83
48	110000			●	●					2	2	1.2	84
64	1000000		●							1	2	1.2	85
144	10010000	●			●					2	2	1.2	86
162	10100010	●		●				●		3	2	1.2	87
187	10111011	●		●	●	●		●	●	6	2	1.2	88
193	11000001	●	●						●	3	2	1.2	90
217	11011001	●	●		●	●			●	5	2	1.2	91
225	11100001	●	●	●					●	4	2	1.2	92
16	10000				●					1	1	0.6	92
34	100010			●				●		2	1	0.6	93
58	111010			●	●	●		●		4	1	0.6	94
96	1100000		●	●						2	1	0.6	94
129	10000001	●							●	2	1	0.6	95
130	10000010	●						●		2	1	0.6	95
132	10000100	●					●			2	1	0.6	96
156	10011100	●			●	●	●			4	1	0.6	97
188	10111100	●		●	●	●	●			5	1	0.6	97
194	11000010	●	●					●		3	1	0.6	98
232	11101000	●	●	●		●				4	1	0.6	98
240	11110000	●	●	●	●					4	1	0.6	99
254	11111110	●	●	●	●	●	●	●		7	1	0.6	99
255	11111111	●	●	●	●	●	●	●	●	8	1	0.6	100
0	0									0	3	1.7	

FIGURE 2. Profiles of IgE sensitization to eight *Phleum pratense* molecules in 176 sensitized children. Profiles of IgE sensitization to eight *Phleum pratense* molecules in 176 children with an IgE reaction to *Phleum pratense*. The Allergen Profile Codification System (APCS) code and the absolute and cumulative frequency are shown. The profiles are ordered by declining frequency and the point at which the arbitrary threshold of 80% of the patient population has been reached is marked in red. Reprinted with permission from [10¹¹].

of IgE sensitization to species-specific or 'genuine' molecules allows discriminating patients with 'true' sensitization from those with sensitization due to cross-reacting pan-allergens [17]. Again, in a series of patients with mite allergy, the lack of Der p 1 and Der p 2 IgE may help with differential diagnosis [18]. We do not know, however, whether patients with more 'complex' profiles of IgE sensitization (e.g. to *Phleum pratense* [10^{***}]) suffer from more severe symptoms or whether IgE sensitization to a given component is a hallmark of specific disease characteristics. These questions will be answered only by larger epidemiological studies. An interesting hypothesis, however, is that the overall concentration of IgE against proteins belonging to the same pollen – that is, an allergenic source impacting directly on its effector site – is more important for symptom severity than their molecular specificity and diversity. Accordingly, bronchial responsiveness and the risk of asthma increase with the number of sensitizations to different allergen groups of molecules [19^{*}]. On the contrary, additional information given by IgE molecular assays is not always considered to be of clinical relevance. For example, it seems that detecting IgE reactivity to a single marker protein (e.g., Bet v 2) is sufficient to diagnose or exclude sensitization to profilin. According to the authors, detecting IgE to multiple homologous, cross-reacting allergen proteins (such as Bet v 2, Ole e 2, Hev b 8, Mer a 1, and Phl p 12) is not clinically more informative and increases the risk of confusion and misinterpretation [20].

DO SENSITIZATION PROFILES AFFECT SPECIFIC IMMUNOTHERAPY OUTCOME?

Personalized or patient-tailored immunotherapy is still a very distant prospect [21], but the first recombinant products based on single allergens or defined mixtures could reach the market within the next 5 years [22]. However, we do not know whether patients with different sensitization profiles respond differently to the same allergen-specific immunotherapy treatment. This question has been recently theoretically addressed by a study [10^{***}] modeling all the different possible combinations occurring between a given IgE molecular sensitization profile and the molecular profile of an allergen SIT preparation previously clinically tested [23]. The two profiles could be perfectly matched or mismatched. Four different categories of mismatching have been described and their immunological and clinical correlations hypothesized (Fig. 3).

In type 1 mismatch ('underpowered' immunization) patients would receive immunization with only some of the molecules they are sensitized to.

The efficacy of the immunization might be insufficient in this category.

In type 2 mismatch ('overpowered' immunization), patients would receive all the molecules to which they are sensitized but also molecules to which they are not sensitized. Three mutually exclusive consequences could be predicted in this category: a beneficial induction of IgG antibodies and/or prevention of new IgE sensitization [23]; an unwanted IgE sensitization to these molecules [24]; and no effect [25,26].

Type 3 mismatch ('underpowered and overpowered' immunization) includes patients with a mixed (type 1 and type 2) mismatch. In this group a combination of the effects described in the previous categories could be expected.

In type 4 mismatch ('unrelated immunization') patients would be immunized with molecules to which they are not sensitized and would not be immunized with the molecules to which they are sensitized. This category would probably be the one with the lowest (if any) benefit and the highest risk of unwanted effects.

The analysis of sera of participants to trials large enough to provide enough statistical power may answer this question. An interesting hypothesis is that, given the synergistic role of individual molecules in triggering IgE-mediated degranulation of Mast-cells, the overall immune-response to SIT may be linked more to the overall concentration of the IgE antibodies directed against a pollen allergenic source than to the nature of the individual molecules that they recognize. The impact of multiple sensitization on the efficacy of SIT has been so far examined only at the level of extracts [27–29]. In polysensitized allergic rhinitis patients, SLIT for *D pteronyssinus* and/or *D farinae* produced improvements in both nasal symptoms and rescue medication scores comparable to those in mono-sensitized patients, regardless of other positive allergens. SLIT for *D pteronyssinus* and/or *D farinae* should be considered in polysensitized allergic rhinitis patients [30].

'MOLECULAR SPREADING' OF THE IGE RESPONSE IN CHILDHOOD

A huge cross-sectional study [31] described the differences of molecular profiles of sensitization in over 23 000 allergic patients stratified by age at examination. This study showed that the prevalence of sensitization to allergenic molecules varies quite markedly depending on the age range considered, suggesting a different behavior in the lifetime sensitization process. Similarly, a recent cross-sectional study [32^{*}] has shown that children are sensitized to

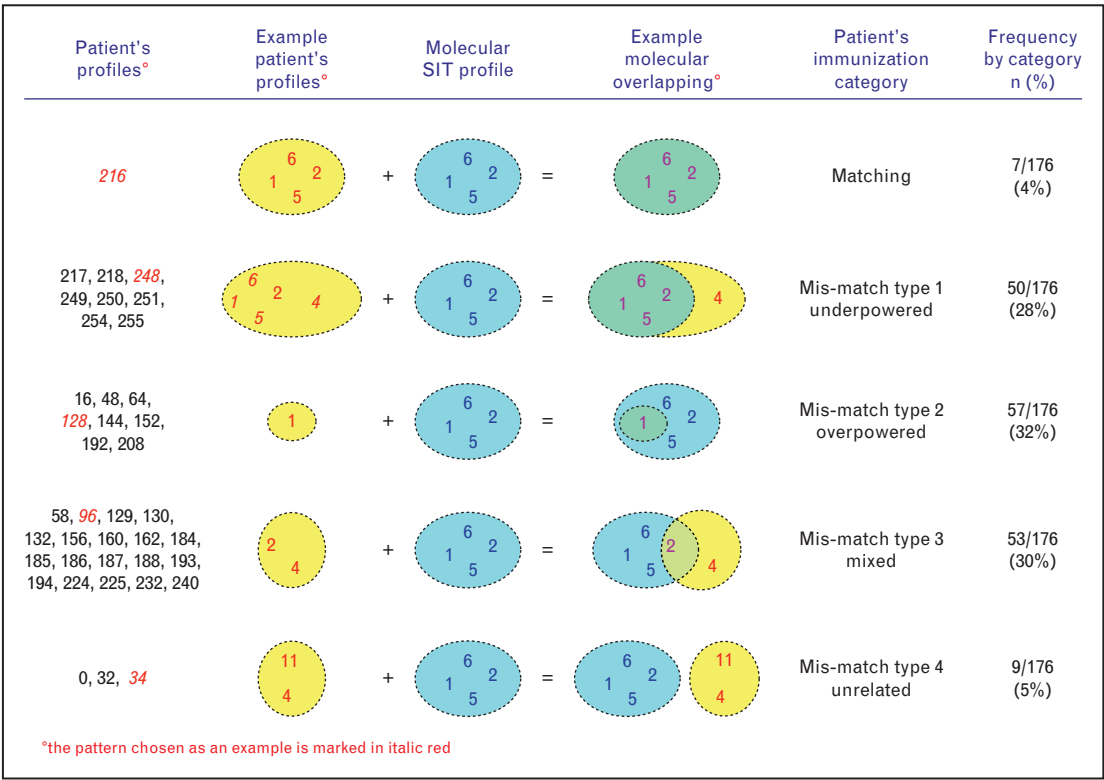


FIGURE 3. Categories of potential molecular matching and mismatching with SIT. Matching molecular profiles of IgE sensitization to *Phleum pratense* against the composition of a molecularly designed SIT preparation in 176 children with grass pollen respiratory allergies. Each of the 39 profiles represented in Figure 1 is here reported. Five categories have been created with all the five different possible typologies of matching/mismatching between sensitization profiles and composition of the SIT preparation. SIT, specific immunotherapy. Reprinted with permission from [10^{¶¶}].

fewer molecules (including milk, egg and mite molecules) than adolescents. In keeping with the concept of the allergic march, specific IgE against highly cross-reacting allergenic molecules were observed only in children aged more than 6 years [32[¶]].

Cross-sectional study cannot establish whether sensitization profiles are a stable hallmark of the individual patient, or whether they dynamically change over time. Interestingly, a recent study [33^{¶¶}] in adult patients has clearly shown that the fingerprint of the IgE response is a stable characteristic of the individual patient. Allergic adults whose serum had been collected in the late 1990s and in 2010 preserved exactly the same sensitization profile in the two follow-up points taken 10 years apart [33^{¶¶}]. By contrast, the IgE response is extremely dynamic during childhood [34^{¶¶}]. In particular, the IgE response against *Phleum pratense* evolves in many allergic children from a simple, often molecular monosensitization stage to an oligomolecular sensitization stage to terminate its evolution in a polymolecular sensitization stage. This phenomenon has been defined as ‘molecular spreading’, in analogy with the epitope spreading phenomenon

described in the T-cell response during the evolution of an autoimmune reaction. Interestingly, the IgE response in over 75% of the grass-pollen German allergic children participating in the Multicenter Allergy Study birth cohort study starts with IgE antibodies recognizing Phl p 1 (a sort of ‘initiator’ of this IgE response). This may imply that Phl p 1 is a valuable target for early intervention [34^{¶¶},35]. Then the response involves as a second molecule Phl p 4 and Phl p 5; thereafter also Phl p 2 and Phl p 6, and finally Phl p 11, Phl p 12, and Phl p 7. Only a few children develop a full blown response with involvement of all the eight allergenic molecules of *Phleum pratense*. However, the IgE response evolves over time and becomes more and more complex. In parallel, the serum concentration of IgE antibodies against grass pollen extracts grows progressively both for an increase in the number of interested molecules, and for an increase of antibodies directed against any individual allergenic molecule (Fig. 4). Interestingly, this ‘allergen march’ occurs mostly in the preclinical stages of sensitization and starts with IgE to Phl p 1 even up to 5 years before the child begins to perceive disease symptoms (Fig. 5) ‘Preclinical’ IgE sensitization, characterized by weak

and simple IgE responses, is progressively increasing in concentration and molecular complexity. A 'clinical' stage of the IgE response, when inflammation is clinically perceived as a disease, is characterized by higher serum levels of grass-specific IgE antibodies and a higher number of molecules involved. In the first years after the onset of allergic rhinitis, a slower increasing trend in the number of molecules recognized by IgE is observed, whereas the overall serum concentration of IgE antibodies against Phleum pratense still grows at a consistent pace [34[■]].

MOLECULAR SPREADING: IMPLICATIONS AND NEW QUESTIONS

An interesting question is whether the preclinical molecular spreading phenomenon is limited to grass pollen or if it is a general characteristic of the IgE responses. The data from the cross-sectional studies reported above suggest that this phenomenon is occurring also in IgE responses against other allergenic sources. We can also postulate that the 'deviation' of the immune response is more susceptible to be corrected and readdressed in the first, weaker, preclinical monomolecular stages, rather

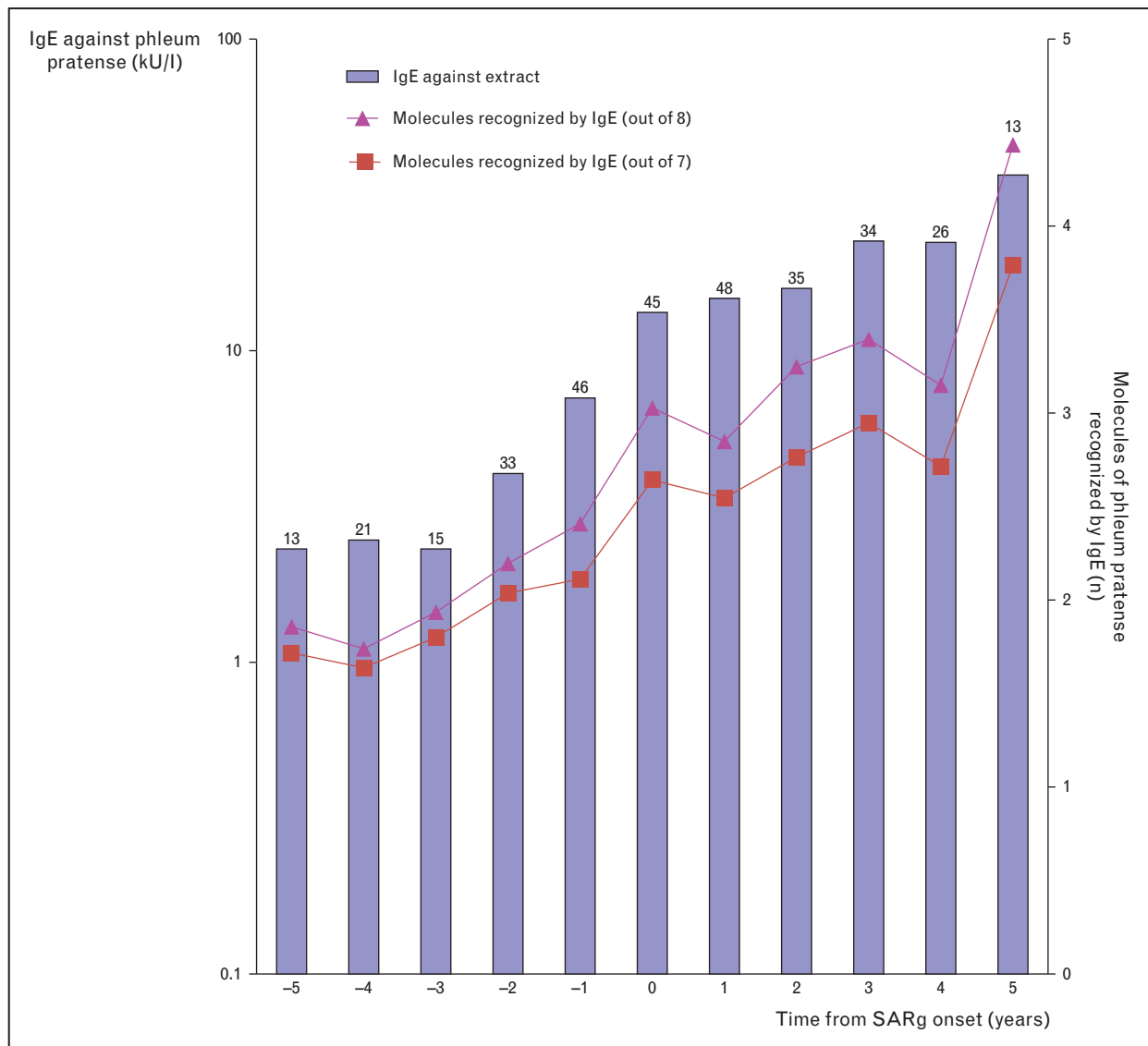


FIGURE 4. IgE to P pratense by time from onset of grass-related seasonal allergic rhinitis. A, Bars show the prevalence of IgE sensitization (>0.35 kUA/L) to P pratense (extract) in children whose sera were available at each point in time. The number of tested children is indicated over each bar. B, Bars show the geometric mean levels of IgE antibodies to P pratense (extract) at each point in time in IgE antibody-positive sera. The numbers of tested children is indicated over each bar. Lines show the average number of all 8 (triangles) or only 7 (squares; Phl p 6 excluded) allergenic molecules of P pratense recognized by IgE antibodies at each point in time in IgE antibody-positive sera [34[■]].

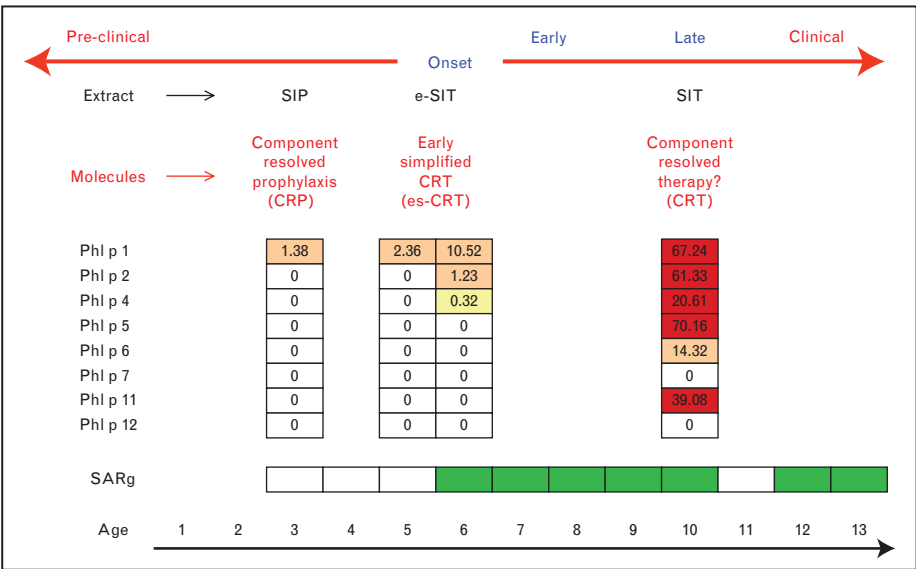


FIGURE 5. Molecular spreading of the IgE response to Timothy grass and potential implications for allergen-specific immunological intervention in a child with seasonal allergic rhinitis to grass pollen. ‘Molecular spreading of the IgE response to *Phleum pratense* and implications for allergen-specific immunological intervention in one child with hay fever (case from the MAS birth cohort). This child started suffering from hay fever symptoms at the age of 6 years. IgE response against *Phleum pratense* started 3 years before with a weak, monomolecular sensitization to Phl p 1. This IgE response was stronger and directed also to Phl p 2 and Phl p 4 at disease onset. After disease onset the IgE response was much stronger and directed also to Phl p 5, Phl p 6, and Phl p 11. In clinical practice, allergen-SIT would be “normally” prescribed at this advanced stage, after some years of symptoms (age 10 years). An interesting hypothesis is that SIT would be more efficient if started much earlier, ideally “at” disease onset (age 6 years) (early SIT). Moreover, it could be investigated whether an immune intervention at the earliest, preclinical stages (age 3 years) of disease could even better change the natural history of the sensitization and prevent or delay disease onset (allergen-specific immunoprophylaxis, SIP). The use of recombinant allergens would be easier at this stage, as less molecules should be used. CRP, component resolved immune-prophylaxis; SARg, seasonal allergic rhinitis to grass; SIT, specific immunotherapy.

than in the advanced, stronger and polymolecular, clinical stages. This suggests that early monitoring may be extremely helpful in starting an allergen-specific immune intervention as soon as possible that, when applied in preclinical stages, would be called ‘allergen-specific immunoprophylaxis’ or immunoprevention. This concept was proposed years ago for primary prevention [36] and for secondary prevention [7,37]. Similarly, given the increase in complexity and concentration of the IgE response after the onset of perceived disease, we can hypothesize that early versus delayed SIT intervention in children with the appropriate characteristics, is more effective and is able to raise the interest of the specialist as well. Early specific immunotherapy and even immunoprophylaxis can be future challenges for clinical research in this area. In addition, given the simplicity of the very first stages of the IgE response to grass pollen, it is not inconceivable that both the immunoprophylactic approach and the early immunotherapeutic approach be based in the future on tailored CRT preparations. In many cases, indeed, the required

preparation would be limited to recombinant Phl p 1 only.

CONCLUSION

In childhood, IgE sensitization to grass pollen starts preclinically as a weak, monomolecular or oligomolecular response and evolves rapidly to become strong, polymolecular, and associated with clinical manifestations. This immunological phenomenon, defined ‘molecular spreading’, suggests that the immune response could be more susceptible to suppression by immune-intervention at earlier rather than later stages of the sensitization process. This hypothesis – proposed many years ago – has gained strong support from recent serological studies and deserves, therefore, to be tested in properly designed clinical trials.

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Conflicts of interest

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