

REVIEW

Immunoglobulin E-binding autoantigens: biochemical characterization and clinical relevance

R. Crameri

Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos, Switzerland

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Summary

Although immediate-Type I skin reactions to human dander have been described six decades ago, only the recent application of molecular biology to allergology research allowed fast and detailed characterization of IgE-binding autoantigens. These can be functionally subdivided into three classes: (1) self-antigens with sequence homology to environmental allergens belonging to the class of phylogenetically conserved proteins, (2) self-antigens without sequence homology to known environmental allergens, and (3) chemically modified self-antigens deriving from workplace exposure. As environmental allergens, also IgE-binding autoantigens belong to different protein families without common structural features that would explain their IgE-binding capability. Many of the self-antigens showing sequence homology to environmental allergens, are phylogenetically conserved proteins like manganese dependent superoxide dismutase, thioredoxin or cyclophilin. Their IgE-binding capability can be explained by molecular mimicry resulting from shared B-cell epitopes. A common factor of IgE-binding self-antigens without sequence homology to known environmental allergens is that they elicit IgE responses only in individuals suffering from long-lasting atopic diseases. In contrast, IgE-mediated reactions to modified self-antigens might be explained with the generation of novel B-cell epitopes. Chemically modified self-antigens are likely to be recognized as non-self by the immune system. The clinical relevance of IgE responses to self-antigens remains largely unclear. Well documented is their ability to induce immediate Type I skin reactions *in vivo*, and to induce mediator release from effector cells of sensitized individuals *in vitro*. Based on these observations it is reasonable to assume that IgE-mediated cross-linking of FcRIε receptors on effector cells can elicit the same symptoms as those induced by environmental allergens, and this could explain exacerbations of chronic allergic diseases in the absence of external exposure. However, because most of the described IgE-binding self-antigens are intracellular proteins normally not accessible for antigen–antibody interactions, local release of the antigens is required to explain the induction of symptoms.

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Prof. Reto Crameri, Department
Molecular Allergology, Swiss Institute
of Allergy and Asthma Research
(SIAF), Obere Strasse 22, CH-7270
Davos, Switzerland.
E-mail: crameri@siaf.uzh.ch

Allergy, immunoglobulin E-mediated autoreactivity and immunoglobulin E-mediated autoimmunity

Allergy, also called Type I hypersensitivity is clearly defined as an immediate reaction occurring a few minutes after allergen exposure [1]. Mechanistically Type I hypersensitivity reactions are due to allergen-mediated cross-linking of allergen-specific IgE bound to high-affinity FcRIε receptors on the surface of effector cells [2]. Cross-linking of the receptors results in immediate release of toxic mediators responsible for the allergen-

induced symptomatic in sensitized individuals [2–4]. IgE-mediated autoreactivity to self-antigens and IgE autoimmunity are less clearly defined and we should distinguish between two subtypes: autoreactivity due to cross-reactive IgE antibodies resulting from the interaction between IgE raised against environmental allergens displaying a high sequence homology to human self-antigens, and IgE antibodies directly raised against self-antigens resulting in ‘true’ autoimmune reactions. A special case of autoreactivity might result from chemical substances which modify self-antigens thus creating

new IgE-binding epitopes normally absent in nature. According to this classification three main sections will be discussed herein: (1) IgE-mediated autoreactivity to cross-reactive allergens, (2) IgE-mediated autoimmune reactions and (3) IgE reactivity to modified self-antigens.

Immunoglobulin E-mediated autoreactivity to cross-reactive allergens

Although Type I sensitivity reactions against human dander have been observed for a long time [5], the biochemical nature of the molecular structures involved in these reactions remained unidentified until progress in molecular allergology allowed cloning, characterization, production and purification of recombinant allergens and autoantigens. The first example of a self-antigen binding serum IgE was profilin, a human self-antigen sharing sequence homology to the birch pollen allergen Bet v 2 [6]. This work showed that serum IgE from birch pollen allergic individuals bound not only to natural and recombinant birch profilin but also to human profilin, indicating that structural similarity of conserved proteins might be responsible for IgE-mediated autoreactivity. Although human profilin was able to induce histamine release from blood basophils of Bet v 2-sensitized individuals [6], attempts to induce positive skin test reactions with human profilin failed [7]. The lack of skin test reactivity to human profilin can have manifold reasons, however, in view of the relatively low sequence identity shared between birch and human profilin a strong cross-reactivity mediated by high-affinity IgE antibodies might not be expected [8–10]. The first clear example that a human self-antigen with high sequence identity to an environmental allergen can induce strong skin test reactions derived from studies aimed at cloning and characterizing *Aspergillus fu-*

migatus allergens to clarify their roles in allergic bronchopulmonary aspergillosis (ABPA), an intense inflammatory lung disease affecting asthmatic and cystic fibrosis patients sensitized to the mould [11, 12]. Human manganese dependent superoxide dismutase (MnSOD), an essential phylogenetically conserved enzyme, shows a high degree of sequence homology to the *A. fumigatus* MnSOD (Asp f 6). Both sequences coding for these MnSODs were cloned, produced as recombinant proteins and tested for their ability to recognize serum IgE and induce skin reactions in patients sensitized to the MnSOD of *A. fumigatus* [13]. Both protein preparations were able to induce strong immediate-type I skin reactions and were recognized by human serum IgE [14]. Cross-reactivity between Asp f 6 and human MnSOD was further confirmed in extended immunological and structural studies [15]. Furthermore, it was clearly shown that T- and B-cell-mediated autoreactivity was not only involved in the pathogenesis of ABPA, but also in severe atopic dermatitis [16, 17]. Cloning of human self-antigens suspected to represent IgE-binding proteins based on their high sequence identity with environmental allergens including acidic ribosomal P₂ protein [18], cyclophilins [19, 20] and thioredoxin [21] showed that all these self-antigens bind IgE from serum of allergic patients sensitized to the corresponding environmental structure and, as a strong indication for the biological relevance of this cross-reactivity, were able to induce strong Type I skin reactions [16–21]. A description of the best identified self-antigens with sequence homology to environmental allergens and their properties is reported in Table 1. To analyse the structural basis of the IgE-mediated reactivity demonstrated *in vitro* and *in vivo*, we solved the 3D structures of the *A. fumigatus* MnSOD [22] and cyclophilin [23] as well as the cyclophilin [20] and thioredoxin [21] of *Malassezia sympodialis*, a lipohylic yeast associated

Table 1. IgE-binding self-antigens with sequence homology to environmental allergens

Protein	GenBank accession number	Amino acids	Molecular weight (kDa)	Biological function	Identity to allergen (%)	Skin test	References
Profilin	NM_005022	140	15	Actin-binding protein	Bet v 2 (34)	Neg	6, 7
MnSOD	NM_000636	198	26.7	Superoxide dismutase	Asp f 6 (51)	Pos	15, 22
P ₂ ribosomal protein	NM_001004	115	13	Functional activity of ribosomes	Asp f 8 (62)	Pos	18, 41
Cyclophilin A	NM_021130	165	17.2	Isomerase	Asp f 11 (60) Mala s 6 (71)	NT	19
Cyclophilin B	NM_000942	216	22.7	Isomerase	Asp f 11 (55) Mala s 6 (63)	Pos	19, 20
Cyclophilin C	NM_000943	212	22.8	Isomerase	Asp f 11 (51) Mala s 6 (60)	NT	19
Thioredoxin	NM_003329	105	12	Oxidoreductase	Asp f 29 (48) Mala s 13 (44)	Pos	21, 27

Neg, negative; NT, not tested; Pos, positive.

with atopic dermatitis [24]. Superimposition of the solved allergen structures with those of the homologous human proteins revealed in all cases large structural similarities with only minor deviations (Fig. 1). To define surface regions potentially involved in IgE-mediated cross-reactivity, we determined shared features on the level of primary and tertiary structures. Although the human and fungal proteins share between 45% and 60% identical amino acids at the primary structure level, only a minority of them are solvent exposed in homologous pairs and, therefore, likely to be accessible for antigen–antibody interactions [20–23]. These amino acids are scattered over the whole linear sequences of the proteins but become clustered on the surface [20–24], forming relevant patches of solvent-accessible surface areas fulfilling the criteria for putative IgE-binding epitopes [25]. Of course the biological relevance of such putative epitopes needs to be experimentally verified. In the case of MnSOD it could be shown that site-directed mutagenesis of amino acids potentially involved in IgE-binding resulted in a considerably lower IgE-binding capacity compared to the wild-type enzyme while conserving the 3D structure [26], indicating a direct involvement of these residues in IgE-mediated cross-reactivity. Indirect evidence for the involvement of

shared amino acid in cross-reactivity was also derived from comparison of the size of the patches shared between different thioredoxins, a pan-allergen family [21]. Analysis of the molecular surfaces combined with sequence alignments allowed identification of conserved solvent exposed amino acids distantly located in the linear sequences which cluster to patches of continuous surface areas. The size of the surface areas conserved between human and fungal thioredoxins correlates well with the inhibitory potential of the molecules in inhibition ELISA indicating that the shared amino acids are involved in IgE-binding [27]. Moreover, it was demonstrated that *M. sympodialis* thioredoxin-specific T cells are highly cross-reactive with human thioredoxin [28]. Interestingly thioredoxin was also identified as a relevant allergen of wheat and maize during repertoire screenings of phage surface displayed libraries with sera from double-blind, placebo-controlled food challenge positive patients [29]. This prompted us to screen the wheat cDNA library against immobilized serum IgE from bakers suffering from occupational asthma and again phage displaying wheat thioredoxin (Tri a 25) were enriched from the library [30]. As expected Tri a 25 cross reacted with other thioredoxins including the human enzyme and competi-

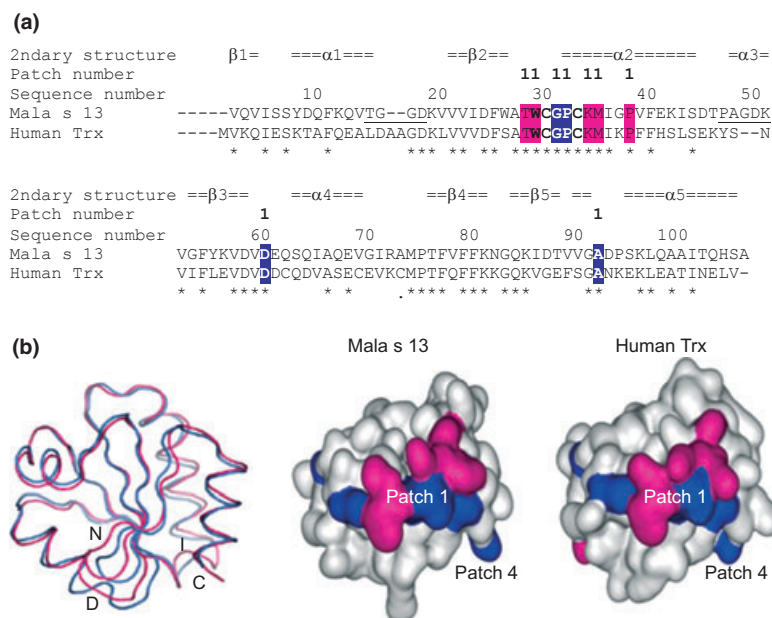


Fig. 1. (a) Structure alignment of Mala s 13 and human thioredoxin. The first line denotes the secondary structure of Mala s 13 assigned by DSSP [89], the second line shows the number and position of identical residues clustering to surface patch 1 shown in (b) and the third line shows the sequence numbering of Mala s 13. Identical amino acids are indicated by an asterisk. The two loops which adopt a different conformation in Mala s 13 compared with human thioredoxin are underlined. Residues that are identical and at last 50% or between 30% and 50% solvent exposed in both enzymes are shown on a pink or blue background, respectively (modified from ref. [21]). (b) Left: superposition of Mala s 13 (pink) on human thioredoxin. D and I denote minor distortions due to a deletion in the first helix and additional short helix due to an insertion. Carboxy- and amino-terminal residues are denoted C and N, respectively. Right: solvent-accessible surfaces of Mala s 13 and human thioredoxin showing identical putative IgE-binding residues which cluster to a continuous surface exemplified for patch 1. Pink and blue colours depict amino acids exposed to the solvent >50% and between 30% and 50%, respectively (modified from ref. [21]).

tion for IgE-binding correlated with the percentage of sequence identity in ELISA inhibition experiments [30], demonstrating that IgE reactivity to self-antigens can also occur in severe asthma without fungal sensitization. These results are sufficient to explain the observed cross-reactivity between self-antigens and environmental allergens by molecular mimicry and the clinical relevance of such reactions has been clearly documented [13, 14, 16, 31–33].

Immunoglobulin E-mediated autoimmune reactions

If genuine IgE-mediated autoimmune reactions against self-antigens really occur, it is still a controversial issue [31] because it is not easy to directly correlate the presence of antigen-specific IgE with the symptoms of the disease. Similarly, in classical allergies to environmental allergens, up to 30% of subjects showing elevated serum levels of allergen-specific IgE are asymptomatic [34]. However, screening of human cDNA libraries either with classical methods [35] or by phage surface display technology [36] with sera of patients suffering from long-lasting chronic allergic diseases revealed a large repertoire of IgE-binding self-antigens [37]. The long list of cDNA's potentially encoding IgE-binding autoantigens covers different classes of proteins and can be tentatively subdivided in the following classes: (1) proteins showing a high degree of sequence homology to environmental allergens normally representing phylogenetically highly conserved proteins; (2) intracellular proteins isolated with sera from atopic dermatitis patients and (3) autoantigens associated with classical autoimmune diseases shown to react with IgE autoantibodies [38].

A special case of IgE-mediated autoreactivity might be represented by human ribosomal P₂ protein, a classical autoantigen in lupus erythematosus [39], which

shows a high degree of sequence homology with eukaryotic P₂ proteins described as environmental allergens [18, 40]. Recently it has been shown that an array of fungal P₂ proteins bind IgE from systemic lupus erythematosus (SLE) patients showing IgG autoantibodies to human P₂ protein in spite of the fact that the patients were not sensitized to any of the fungi or other environmental allergens [41]. Interestingly 31% of the patient's sera showing specific IgG against the human P₂ protein also showed strong IgE responses to this self-antigen. It is therefore tempting to speculate that the P₂-specific IgE in SLE patients could derive from a genuine autoimmune reaction in an environment where B cells are prone to switch towards the production of autoantibodies against self-antigens. Notably, the incidence of SLE patients showing IgG responses to P₂ acidic ribosomal protein switching to IgE reflects more or less the incidence of atopy in the normal population [42]. Although, the clinical relevance of these reactions remains to be elucidated, a recent study clearly shows that individuals with SLE have elevated serum IgE levels, self-reactive IgEs and activated basophils expressing CD62 ligand and the major histocompatibility class II complex. These parameters are associated with increased disease activity and active lupus nephritis [43] arguing that genuine IgE autoantibody responses are involved in the development of lupus nephritis.

The best investigated autoantigens without sequence homology to environmental allergens are reported in Table 2. A first difficulty for the interpretation of the relevance of IgE-mediated autoimmune reactions derives from the fact that the repertoire of environmental allergens, although supposed to be limited [44, 45], is far from complete. Therefore, we cannot exclude that these reactions could be due to molecular mimicry induced by thus far undetected environmental allergens. We can assume, however, that the rapid progress in

Table 2. IgE-binding self-antigens without sequence identity to environmental allergens

Protein	EMBL accession number	Amino acids*	Molecular weight (kDa)	Functional test†	Tissue distribution	References
Hom s 1	AB006198	800	73.4	SPT	Intracellular	35, 38, 92
Hom s 2	AJ278883	215	23.2	SPT	Intracellular	35, 38, 93
Hom s 3	X89985	202	20.1	SPT	Intracellular	35, 38
Hom s 4‡	Y17711	476	54.2	SPT	Intracellular	35, 38, 46
Hom s 5	BC069269	564	42.6	SPT	Intracellular	35, 38
Actin-α	NM_001613	377	42	BAT	Intracellular	37, 53
Tubulin-α	NM_006009	451	50	BAT	Intracellular	37, 53
eIF6	NM_002212	245	27	BAT	Intracellular	37, 94
HLA-DR-α§	NM_019111	254	24	BAT	Cell surface	37
RP1	NM_014268	327	39	BAT	Intracellular	37

*Predicted from the nucleotide sequence.

†Functional test indicative for *in vivo* relevance. SPT, skin prick test; BAT, basophil activation test.

‡Hom s 4 shows homology to calcium-binding allergens.

§Recombinant HLA-DR-α protein was unable to activate basophils from atopic dermatitis patients showing serum IgE raised against the protein.

both discovering these undetected environmental allergens and in genome sequences will help clarify if autoantigens identified as IgE-binding proteins share sequence homology with environmental allergens. A good example thereof is ara-the calcium binding protein C (CALC), an IgE-binding self-antigen initially claimed to lack sequence homology to environmental allergens [32]. Later on re-named to Hom s 4, it was demonstrated that CALC cross reacts with environmental allergens belonging to the well-known family of CALC [46, 47]. A second (major) problem is due to the fact that not all proteins shown to bind IgE *in vitro* are clinically relevant. Just to mention an example: human leukocyte antigen-DR- α , isolated as an IgE-binding protein from a human cDNA library displayed on phage surface was unable to activate basophils from blood of atopic dermatitis patients with specific IgE against the self-antigen [37]. The demonstration that a self-antigen can bind serum IgE from allergic patients is, therefore, not sufficient to claim that the proteins represent an auto-allergen.

However, at least for the autoantigens mentioned in Table 2, direct or indirect evidence of clinical relevance has been reported. Direct evidence for a potential *in vivo* relevance of autoallergens can be derived from their capability to induce immediate skin test or positive atopy patch test reactions [48, 49]. Indirect evidence can be derived from basophil activation tests [50], *in vitro* induction of mediator release [51] or from the ability of autoallergens to induce specific T cell proliferation in sensitized individuals [52]. In any case, even if these tests are strongly positive this does not mean that they have direct consequences for the patients. In fact the majority of the so far described IgE-binding self-antigens, independent of shared sequence homology to environmental allergens, are intracellular proteins and are therefore normally not accessible for antigen-antibody interactions *in vivo*. For interactions with antigen-specific IgE, and thus to elicit symptoms, intracellular proteins need to be released from the cells. There is good evidence showing that IgE-binding autoantigens are over-expressed in lesional skin of atopic eczema patients [33, 53] and, under inflammatory conditions, also released [54–56]. Secondary immune responses might be boosted by autoantigens released under inflammatory conditions [54] or diminished in the absence of antigen exposure [57] explaining the correlation found between IgE autoantibody levels and disease activity and severity found in patients suffering from atopic dermatitis [35]. However, the precise role of IgE-mediated autoimmune reactions in long-lasting chronic allergic diseases still remains to be elucidated. In contrast there is cumulating evidence showing that IgE-mediated autoimmune reactions could play a decisive role in the pathogenesis of different dis-

eases. It is well accepted that urticaria patients can be stratified into suffering from autoimmune- and idiopathic chronic urticaria [58]. The autoimmune subgroup is associated with IgG and IgE responses raised against the high-affinity receptor for IgE [59] and against different thyroid self-antigens [60–62]. The perhaps more direct evidence that IgE-mediated autoimmune reactions play a role in the pathogenesis of spontaneous chronic urticaria, although needed to be confirmed in larger clinical studies, derives from the observation that Omalizuman-based anti-IgE therapy significantly improved the clinical status in a limited number of patients suffering from therapy-resistant spontaneous chronic urticaria [63]. The involvement of IgE-mediated autoimmunity has been postulated for several other autoimmune diseases including rheumatoid arthritis [64], type 1 diabetes mellitus [65], coeliac disease [66], pemphigus vulgaris [67] and bullous pemphigoid [68]. For bullous pemphigoid there is large evidence from mouse models for a pathogenic role of IgE-mediated autoimmune reactions in the development of skin lesions [68–70]. These observations are strongly supported by a case report describing the successful treatment of bullous pemphigoid with omalizumab [71], a humanized monoclonal antibody that inhibits IgE binding to its high-affinity receptor [72]. Although for most of these IgE-reactive autoantigens it has not been demonstrated that the IgE reactivity contributes to the pathology of the disease or disease severity, we can assume that the repertoire of IgE-binding self-antigens associated with classical autoimmune diseases is much larger than presently assumed. The simple reason for this assumption is that we detect IgE-binding to an antigen only if we are looking for it and to perform binding assays it is necessary to have the purified antigen and sera of patients suffering from autoimmune diseases.

Immunoglobulin E reactivity to modified self-antigens

IgE-mediated reactions to modified self-antigens are poorly investigated. They can be considered as pseudo autoimmune reactions and there is a body of evidence suggesting that under certain circumstances they could play an important role. A classical example thereof is the high incidence of insulin allergy in patients treated with swine or bovine insulin [73]. Although they differ only in a few amino acids compared to human insulin, they are recognized by the immune system as foreign proteins and have been reported as allergens [74]. The introduction of recombinant human insulin has dramatically reduced the prevalence of hypersensitivity reactions [75] but even if rare, allergy to recombinant human insulin still represents a serious problem for affected individuals [76]. Altered immunogenic proper-

ties of insulin might result from aggregation [77, 78] or small differences in amino acid composition between different therapeutic preparations [79] highlighting the plasticity of the immune system in detecting even small changes in protein conformations. In addition, it is well known that modification of proteins with low molecular weight chemicals (haptens) can render them allergenic [80–82]. Haptens are defined as small non-immunogenic molecules that can become sensitizing when they become covalently bound to a protein rendering them immunogenic [82]. The best known haptens associated with occupational asthma are isocyanates [83]. Diisocyanates, widely used for the production of polyurethane-based products are a leading cause of occupational asthma world-wide and can induce specific IgE responses in exposed workers [84]. They react rapidly with functional groups like amines, hydroxyls and sulphhydryls present in proteins under physiological conditions, generating modified proteins [85]. Human serum albumin has been the primary protein studied as a carrier for diisocyanates in most immunological studies [86, 87] and a direct demonstration that human self-proteins, including keratins and albumin, can be modified by exposure to diisocyanates was obtained by investigation of bronchioalveolar lavage from exposed subjects [88]. However, other aggressive chemicals are capable of modifying human proteins after exposure and might result in specific IgE responses against the self-antigens [89, 90]. According to our present understanding haptenization of self-antigens creates novel pseudo epitopes on the modified self-antigens which can elicit specific IgE responses in predisposed individuals.

Conclusions

The data so far available indicate that IgE-mediated reactivity to self-antigens could represent an important pathomechanism in subsets of patients suffering from

chronic long-lasting allergic diseases like atopic dermatitis or asthma bronchiale. Still unclear is if IgE-mediated reactions can be classified as genuine autoimmune reactions or if they represent epiphenomena. In the case of self-antigens sharing a high degree of sequence homology/identity to environmental allergens there is excellent evidence for the involvement of autoreactivity in the pathogenesis of chronic forms of atopy explainable by molecular mimicry. The interpretation of IgE-mediated autoimmune reactions to self-antigens without sequence homology to human proteins is far more complicated. Because the repertoire of environmental allergens is still largely unknown, we do not know at present if IgE-binding self-antigens are present in the environment as homologues of orthologue allergens in nature. The fast progress in genome sequencing together with the fast-growing bioinformatics technology will help to identify both additional self-antigens with sequence homology to environmental allergens and *vice versa*. More work will be required to investigate the effects of allergen-specific immunotherapy using proteins with sequence homology to self-antigens. Allergen-specific immunotherapy is a powerful therapeutic intervention able to cure allergic diseases [85, 91], but the risk of inducing autoimmune diseases through increased IgG responses against self-antigens with sequence homology to environmental allergens still remains to be investigated.

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