Received: 13 September 2019 | Revised: 6 November 2019 | Accepted: 12 November 2019

DOI: 10.1111/all.14120

EDITORIAL

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

Cross-reactivities of non-homologous allergens

Abstract

Allergen cross-reactivities occur when IgE antibodies, originally raised against a specific allergen, bind to identical or highly similar surface areas of another related allergen. It is a commonly held view that cross-reactivity requires more than 70% sequence identity, while proteins that share <50% sequence identity are rarely cross-reactive. This also implies that cross-reactive proteins have a similar 3D fold and belong to the same protein family. At first, the existence of cross-reactivity between non-homologous allergens was not expected because it was contrary to the above described concepts. Now, several lines of evidence demonstrate that IgE cross-reactivity also exists between unrelated allergens. In this Editorial, we aim to summarize the existing literature on IgE cross-reactivity between non-homologous allergens.

Allergen cross-reactivities occur when IgE antibodies, originally raised against a specific allergen, bind to identical or highly similar surface areas of another related allergen. These cross-reactivities are largely determined by secondary and tertiary structural similarities between allergens. It is a commonly held view that cross-reactivity requires more than 70% sequence identity, while proteins that share <50% sequence identity are rarely cross-reactive. This also implies that cross-reactive proteins have a similar 3D fold and belong to the same protein family.[1] An exception is IgE cross-reactivity that is based on conserved structures of cross-reactive carbohydrate determinants, which is not discussed in this Editorial.

The information about potential cross-reactivity is important for allergy diagnostics, the safety and efficacy of allergy vaccines and the risk assessment of novel foods. As part of an allergenicity assessment, linear sequence alignments are frequently used to compare amino acid sequences of query proteins with sequences of known allergens to predict potential cross-reactivities. One of the main criteria to predict cross-reactivity is a search for >35% identity over a window of 80 amino acid residues between a query protein and all known allergens.[2]

At first, the existence of cross-reactivity between non-homologous allergens was not expected because it was contrary to the above described concepts. Now, several lines of evidence demonstrate that IgE cross-reactivity also exists between unrelated allergens (Table 1). In this Editorial, we aim to summarize the existing literature on IgE cross-reactivity between non-homologous allergens. It should be noted that when analyzing cross-reactivity by inhibition assays using purified natural allergens, there is a risk that the preparation is contaminated with other major allergens. Therefore, the cross-reactivity should also be confirmed by other approaches such as using recombinant allergens or cross-reactive antibodies as described below for soy and cow's milk allergens or peanut allergens. The most extensively studied example is the cross-reactivity between bovine casein and soy allergens (see ref.[2] and Table 1). These studies were prompted by frequently reported incidences of allergic reactions to a soy protein formula in cow's milk allergic patients. Monoclonal antibodies specific to α-casein, β-casein, and κ-casein as well as IgE from cow's milk allergic children were reported to recognize the recombinant soy allergen Gly m 6.0401.[3] Additional cross-reactivities were described between the Gly m 5.0101, Gly m Bd30K/34, and Gly m Bd28K, and bovine casein Bos d 8 (Table 1). In a mouse model of food allergy, the authors demonstrated that mice orally sensitized to cow's milk proteins developed cow's milk protein-specific IgE and IgG1 antibodies that cross-reacted with the recombinant soy proteins (Table 1). In addition, the mice displayed positive cutaneous tests to the three recombinant soy allergens and developed allergic symptoms immediately following the exposure to these allergens (Table 1). These in vivo experiments strongly suggested the physiological relevance of the cross-reactivity between unrelated allergens.

IgE cross-reactivity between non-homologous allergens from lupine and peanut has also been reported.[4] Using six lupine and peanut allergic patients' sera, the inhibitory capacity of the peanut allergens Ara h 1, Ara h 2, and Ara h 3 on IgE-binding to lupine α- and δ-conglutins was determined. IgE-binding to the lupine α-conglutin, a cupin, was strongly inhibited by the peanut allergen Ara h 2, a 2S albumin, illustrating IgE cross-reactivity among unrelated allergens. Ara h 1 and Ara h 3, both cupin type allergens, showed similar inhibitions albeit at higher concentrations. Similarly, IgE-binding to the lupine δ-conglutin, a 2S albumin, was inhibited by all tree peanut allergens.

Maleki and colleagues showed IgE cross-reactivity between the walnut cupin Jug r 2 and the peanut 2S albumin Ara h 2.[5] The IgE cross-reactivity between peanut and walnut allergens was studied by testing sera of peanut- and walnut-allergic patients for IgE-binding to peptides representing predicted walnut and known and predicted peanut Ara h 2 epitopes. A predicted Jug r 2 epitope not only bound IgE from the sera but also inhibited IgE-binding to the Ara h 2 and to a known IgE epitope from Ara h 2.[5]

TABLE 1 Overview of cross-reactive non-homologous allergens

Cross-reactive non-homologous allergens	Methods (M) and Results (R)	References
Gly m 6.0401 (soy legumin) and Bos d 8 (bovine caseins)	M: Immunoblotting and competitive ELISA assays. R: Gly m 6.0401 was recognized by bovine casein-specific mAbs and by IgE from cow's milk allergic patients.	Rozenfeld, Clin Exp Immunol.2002;130:49
Gly m 6.0401 (soy legumin) and Bos d 8 (bovine caseins)	M: Production of recombinant rGly m 6.0401, immunoblotting, and ELISA. R: rGly m 6.0401 was recognized by mAbs specific to α-, β-, and κ-casein and by IgE from 93% of cow's milk allergic patients.	Curciarello, Clin Exp Allergy 2008;38:1559
rGly m 5 soy vicilin (β-conglycinin α subunit) and Bos d 8 (bovine caseins)	M: Production of rGly m 5 (α-subunit), a truncated fragment containing the C-terminal domain (α-T) and peptides of α-T; epitope mapping with an overlapping peptide assay; immunoblotting; ELISA; cow's milk allergy mouse model. R: Four cross-reactive IgE epitopes were identified in the α-T domain. Cow's milk sensitized mice had positive cutaneous tests to rGly m 5 (α) and α-T fragment.	Curciarello, Clin Exp Allergy 2008;38:1559 Curciarello, PlosOne 2014;9:e82341
rGly m 5.0101 and Bos d 9.0101 (α-casein)	M: Trypsin digested peptides of rGly m 5.0101 and purified Bos d 9.0101 were used for mapping of cross-reactive epitopes recognized by α-casein-specific mAbs. R: Four peptides of α-casein and three peptides of Gly m 5 with a common core motif were identified.	Candereva, Proteomics 2017;17:15
Gly m Bd 30K/P34 and Bos d 8 (cow's milk caseins)	M: Production of rGly m Bd 30K/P34; immunoblotting; competitive ELISA assay; BAT; cow's milk allergy mouse model. R: rGly m Bd 30K/P34 was recognized by IgE from 9 of 10 cow's milk allergic patients and by α-casein mAbs. It induced activation of basophils sensitized with IgE from milk allergic patients and induced a positive cutaneous reaction and allergic symptoms in milk allergic mice.	Candereva, Allergy Asthma Immunol Res. 2015;7:60
Lupine α-conglutin (legumin) and Ara h 1, Ara h 2 and Ara h 3	M: IgE ELISA inhibition assay with sera from lupine and peanut allergic patients and purified allergens. R: IgE-binding to α-conglutin was inhibited by Ara h 2 by app. 50% at a conc. of 0.01 μg/mL. Ara h 1 and Ara h 3 showed similar inhibition rates at higher concentrations (1 and 10 μg/mL).	Dooper, J Investing Allergol Clin Immunol. 2009;19:283
Lupine δ-conglutin (2S albumin) and peanut allergens Ara h 1, Ara h 2 and Ara h 3	M: IgE ELISA inhibition assay with purified allergens and sera from lupine and peanut allergic patients. R: IgE-binding to δ-conglutin was inhibited with 0.01 μg/ml Ara h 2 by app. 90%, whereas Ara h 1 and Ara h 3 showed similar inhibition rates at higher concentrations (1 and 10 μg/ml).	Dooper, J Investing Allergol Clin Immunol. 2009;19:283
Peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6	M: IgE ELISA inhibition assay with sera from 10 peanut allergic patients sensitized to all four allergens; immunoblotting. R: High degree of IgE cross-reactivity among the four allergens was detected. Ara h 2 IgE-binding peptides similar to peptides in the other three allergens were identified.	Bublin, J Allergy Clin Immunol. 2013;132:118
	M: B- and T-cell responses were analyzed in mice immunized and challenged with purified Ara h 1, Ara h 2, Ara h 3, and Ara h 6. R: Cross-reactivity was observed at the IgE- and the T cell level, but only Ara h 2 and Ara h 6 were able to induce mucosal mast cell degranulation and histamine release.	Smit, Clinical and Translational Allergy. 2015;5:13
Peanut allergens Ara h 1, Ara h 2, and Ara h 3	M: Single-cell RNA sequencing of peripheral blood B cells from six patients with peanut allergy was performed, and each cell's gene expression, splice variants, and antibody sequences were delineated. Six of the IgE antibodies were cloned and expressed. R: Certain IgE antibodies manifested identical gene rearrangements in unrelated individuals. These IgE antibodies showed high affinity and cross-reactivity to the three peanut allergens.	Croote, Science 2018;362:1306
Jug r 2 (walnut vicilin) and Ara h 2 (peanut 2S albumin)	M: In silico prediction of IgE cross-reactive epitopes in Jug r 2 and Ara h 2; testing of predicted peptides for IgE-binding and ELISA inhibition assay. R: A walnut IgE-binding peptide competed for IgE-binding to Ara h 2.	Maleki, Allergy 2011;66:1522

In our study, we found that members of the unrelated protein families of the 2S albumins and the cupins in peanut were highly cross-reactive due to the presence of highly similar sequence stretches present on surface-exposed loops (Figure 1).[6] IgE cross-reactive between the cupins Ara h 1 and Ara h 3 and the 2S albumin Ara h 2 comprised the major fraction of IgE-specific for these allergens and had the highest affinity to Ara h 2. Very recently, Deak and colleagues engineered molecules called covalent heterobivalent inhibitors, designed to specifically and irreversibly bind to IgE that recognizes the two cross-reactive epitopes identified in our study.[7] Remarkably, these molecules designed to inhibit only one immunodominant epitope of Ara h 2 (DPYSPOHSDRRGAGSS) and one of Ara h 6 (QDRQ) yielded an almost complete inhibition of degranulation responses to peanut extract in cellular assays with patients' sera.[7] Notably, the analysis of antibodies expressed by peanut

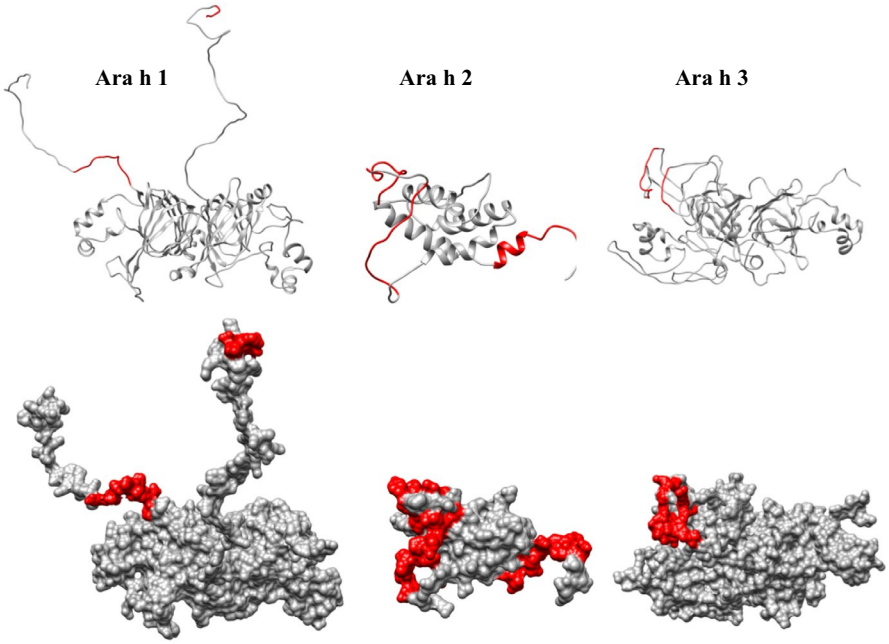

FIGURE 1 Localization of cross-reactive peptides (marked in red) on the structures of Ara h 1 (PDB: 3SMH), Ara h 2 (PDB: 3OB4), and Ara h 3 (PDB: 3C3V). Nonstructured loops missing in the crystal structures were added with Modeller 9v3. Graphics were generated with the molecular modeling system UCSF Chimera (https://www.cgl.ucsf.edu/chimera/)

allergen-specific B-cells in human subjects showed that certain IgE antibodies manifested identical gene rearrangements in unrelated individuals and confirmed the presence of the IgE antibodies with high affinity and cross-reactivity to the three peanut allergens.[8] IgE- and T-cell cross-reactivities between these three allergens were also observed in mice immunized and challenged with purified Ara h 1, Ara h 2, and Ara h 3.[9] The available literature and especially the recent findings on the cross-reactivities between unrelated proteins and the existence of such cross-reactive IgE antibodies underscore the need for a refined analysis of allergen-antibody interactions. The current findings suggest that such cross-reactivities occur frequently among members from different protein families of seed storage proteins and can also occur between seed storage proteins and mammalian proteins. No data are yet available on inhalative allergens. Such studies may reveal a number of surprises that will change our view of the IgE-allergen interactions. A very recent study even provided evidence that the walnut allergen Jug r 2, a cupin, could initiate the development of autoantibodies in individuals genetically predisposed to pemphigus vulgaris.[10]

ACKNOWLEDGMENTS

Author MB acknowledges support by funds of the Oesterreichische Nationalbank (Austrian Central Bank, Anniversary Fund, project number: 17560). Author HB acknowledges the support of the Austrian Science Fund (FWF) Doctoral Program MCCA W1248-B30.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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