Food Research International 54 (2013) 1033–1041

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Food allergen profiling: A big challenge

Maria Antonietta Ciardiello [a], Maurizio Tamburrini [a], Marina Liso [b], Roberta Crescenzo [a,b], Chiara Rafaiani [b], Adriano Mari [b,*]

[a] Institute of Protein Biochemistry, CNR, Naples, Italy
[b] Center for Molecular Allergology, IDI-IRCCS, Rome, Italy

ARTICLE INFO

Article history:
Received 17 October 2012
Received in revised form 28 February 2013
Accepted 8 March 2013

Keywords:
Allergen profile
Food allergen
Microtechnology
Microarray
Proteome
Methodological bias

ABSTRACT

Food allergy is an important public health and safety concern. The description of the pattern of individual allergens associated to each food is of crucial importance both for the diagnostic process and the setting of a safe diet for each allergic subject. However, we are still far from this goal as can be seen from allergen data resources like Allergome. Although some advances have been made during the last few years leading to the identification of new allergens in many allergenic sources and to their characterization, this knowledge is still fragmentary and does not allow the definition of a comprehensive pattern. The exploitation of new technologies and the improvement of those already existing has provided significant contributions, although the widespread use of specific technologies and/or methodologies without cross-checks between them may have generated some biases leading to the preferential identification of selected subsets of allergenic molecules. It would be now desirable to adopt new strategies based on a dynamic combination of different methodologies, spanning from the classic biochemistry-based ones to the innovative microtechnologies and bioinformatics, in order to obtain the best results and give a forward thrust to knowledge in this field.

1. Introduction

Food allergy is widely reported to be on the rise (Fishbein & Fuleihan, 2012; Krause et al., 2002; Linneberg et al., 2000; Maziak et al., 2003; Nicolaou, Siddique, & Custovic, 2005; Sicherer, Munoz-Furlong, Godbold, & Sampson, 2010), but it is actually very difficult to have exact epidemiological data on this topic. The causes of the increase and spread of allergic reactions are still unclear and may be due to a combination of different factors (Fishbein & Fuleihan, 2012; Kemp & Bjorksten, 2003; Mari, 2004; Rottem, 2003; Rottem, Gershwin, & Shoenfeld, 2002). Food allergy can cause one or more symptoms that can be more or less severe and include angioedema, oral allergic syndrome, urticaria, abdominal pain, diarrhea, nausea, vomiting, itching and even life-threatening reactions, such as anaphylactic shock. Therefore, due to the increasing prevalence of allergic reactions and to the effect they have on the quality of life of allergic subjects, the appropriate management of these patients represents a growing public health concern.

As immunotherapy for food allergy is still far from being routinely available (Zuidmeer-Jongejan et al., 2012), the best treatment for food allergic subjects is still based on the avoidance of the allergen source, that means the exclusion of specific foods from the diet. The implication is that a proper management of these patients depends on a reliable diagnosis providing a list of specific allergenic molecules/sources that each subject has to avoid. A reliable diagnosis of food allergy is important not only for the avoidance of foods that may cause a reaction, but also to avoid unnecessary dietary restrictions that may negatively affect the quality of life. In addition, an unequivocal diagnosis is also essential before considering the possibility of future immunotherapeutic treatments (Sastre, Landivar, Ruiz-Garcia, Andregnette-Rosigno, & Mahillo, 2012; Zuidmeer-Jongejan et al., 2012). However, the diagnostic systems now available sometimes fail in their aims and do not provide reliable responses.

The allergy testing methods are based on the detection of specific IgE and/or on the IgE-mediated patient's reaction to the hypothesized allergy source that can be used as a reagent (i) just as it is, or (ii) in the form of a protein extract, or (iii) as individual purified allergens. In the first option, the raw food is used to challenge the allergic subject by *in vivo* tests (prick-by-prick, food challenge), but sometimes these cannot be applied due to different types of limitations. The traditional, and still most common, allergy testing methods are based on the use of commercially available raw protein extracts derived from allergy sources. Protein extract preparations can be used to perform *in vitro* serological tests (Radioallergosorbent test, RAST; ImmunoCAP) and *in vivo* tests (skin prick test, SPT). However, it is well known that the allergen composition of extracts is very variable and their standardization appears impossible (Curin et al., 2011; Focke, Marth, & Valenta, 2009; Hildebrandt, Steinhart, & Paschke, 2008; Kottapalli et al., 2008; Larsen & Dreborg, 2008; Matthes & Schmitz-Eiberger, 2009; van Kampen et al., 2009). Quite recently, the use of individual purified

* Corresponding author at: Center for Molecular Allergology, IDI-IRCCS, Via dei Monti di Creta 104, 00167, Rome, Italy. Tel.: +39 06 6646 4272.
E-mail address: adriano.mari@idi.it (A. Mari).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.03.013

allergen preparations has been introduced into the clinical routine allowing the so-called allergenic molecule-based diagnosis by *in vivo* (SPT) or *in vitro* tests such as Immuno Solid-phase Allergen Chip (ISAC) system (Phadia Multiplexing Diagnostics, PMD, Vienna, Austria) (Bublin et al., 2011; Deinhofer et al., 2004; Harwanegg & Hiller, 2005, 2006; Harwanegg, Hutter, & Hiller, 2007) and Allergenic Molecule-based micro-bead Array system, ABA (Pomponi et al., 2012). Clearly, in the third case, the reagents for molecule-based diagnosis allow a better standardization of the tests than the protein extract-based reagents.

Usually, data from more than one test system are comparatively evaluated in the attempt to reach a diagnosis which is as accurate as possible. False responses can be ascribed to different factors. Probably, the factor that most significantly affects the diagnostic response is the set of allergenic components, as well as their concentration, present in the reagent used to make the test. A false negative result could be due to the lack of one or more allergens in the reagent used for testing, whereas a false positive response could be due to possible contaminations of a food by other foods or by molecules deriving from non-food materials (Anibarro, Seoane, & Mugica, 2007; Polimeno et al., 2010; van der Veen et al., 1996). Ideally, a reagent used for the diagnosis of an allergy to a specific food should exactly contain all the potential allergens (all together in the raw food and extracts, or separated into individual purified allergens) of that food, and nothing more. To achieve this aim, two conditions should be fulfilled: (i) the entire profile of allergenic molecules contained in the allergenic sources should be known, and (ii) reliable protocols and methodologies, useful to assess the pattern of allergenic components really contained in the reagents used by the allergy test systems, should be available.

2. Known food allergens

Many allergens have been identified so far in different allergenic sources, as detailed in the Allergome database (www.allergome.org) (Mari, Rasi, Palazzo, & Scala, 2009; Mari et al., 2006), where 2415 allergenic molecules were listed as of October 15, 2012. Among the reported allergenic sources and individual molecules, those for which a sufficient immunological characterization is available have been officially named by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee (www.allergen.org). Searching in the database of the named allergens that are officially recognized by the WHO/IUIS Sub-committee revealed that overall 740 allergens were listed on September 7, 2012. Among them, 260 molecules (35%) were labeled as food allergens, belonging to the Animalia (phyla: Arthropoda, Chordata, Mollusca, Nemata) and to the Plantae (phyla: Liliopsida and Magnoliopsida) kingdoms (Table 1). Twelve allergens (4.6% of known food allergens) are not real food molecules because they are all proteins from *Anisakis simplex* (Armentia et al., 2006; Audicana & Kennedy, 2008; Kobayashi et al., 2011; Lopez & Pardo, 2011; Vidacek et al., 2010), a parasitic nematode that can infestate some fish species. About 40% of the known food allergens belong to the species reported in Fig. 1, showing the organisms for which 5 or more food allergens have been described.

Table 1
Number of allergens found in different phyla and officially named by the WHO/IUIS.

Kingdom	Phylum	Total allergens	Food allergens
Animalia	Arthropoda	204	28
	Chordata	66	34
	Cnidaria	1	0
	Mollusca	3	3
	Nemata	14	12
Fungi	Ascomycota	82	0
	Basidiomycota	23	0
Plantae	Coniferopsida	14	0
	Liliopsida	84	25
	Magnoliopsida	249	158

Data were collected on September 7th, 2012.

3. The available allergen profiles of food allergenic sources

The description of the allergen profile of a food implies the identification of all the potentially allergenic molecules contained in it. This statement takes for granted the concept that the potential allergenic molecules represent a finite number of proteins, and the remaining components of the proteome of the allergenic source lack the features that cause the activation of the immune system leading to the allergic reaction.

Since the allergenic source may contain more than one allergen, an in-depth characterization is required to describe the complete allergome by classifying as “allergenic” or “not allergenic” the proteome components. However, the available knowledge does not provide sufficient data to classify any food protein as “definitely not allergenic” (Fig. 2). In fact, some proteins appearing not allergenic when a few subjects were analyzed, were recognized as allergens when a larger population, or a population selected on the basis of different criteria, was investigated. In this regard, the kiwifruit allergen Act d 11 can be considered as an example. In fact, initially it appeared non allergenic when it was tested on a population of subjects showing severe reactions after kiwifruit ingestion. However, this protein was identified as an allergen when it was tested on a population of subjects reacting to the birch pollen allergen Bet v 1, thus revealing its immunological correlation with the Bet v 1-like allergens (D'Avino et al., 2011). Indeed, the need for data from a very large, and possibly random, population arises from the observation that single allergic subjects display individual patterns of sensitivity to allergenic molecules.

At present several allergenic sources have been more or less investigated, but none has received an in-depth and targeted characterization allowing the classification of at least most of the proteome components as “allergenic” or “not allergenic”. Ideally, the availability of a panel of individual components of an allergenic source proteome would allow the identification of the complete pattern of allergenic molecules by probing them with individual sera from a very large population of allergic subjects. Indeed, the knowledge and the technologies now available do not yet allow the achievement of this goal.

4. IgE-binding of allergenic and non-allergenic food components

The word “allergy” is generally used to indicate the type I hypersensitivity, that is an IgE-mediated immediate reaction. Non-IgE mediated adverse reactions to food molecules can also occur, but this topic is beyond the scope of this review.

The presence in a patient's serum of IgE antibodies specifically recognizing one, or more, protein molecules contained in an allergy source is exploited in the process of allergy diagnosis and in the detection of allergenic molecules. In fact, the detection in human serum of IgE specific for a given molecule is considered the first clue suggesting the allergenicity of that molecule. Unfortunately, this is not sufficient to classify a molecule as a “real allergen” because some IgE-binding proteins have no clinical relevance since they do not cause any allergic symptoms. For instance, plant proteins bearing N-linked oligosaccharides are recognized by IgE specific for glyco-epitopes, known as cross-reactive carbohydrate determinants (CCD) (van Ree, 2002). The structure of these oligosaccharides is at least partially conserved in a large variety of plant and insect glycoproteins and represents an important cause of co-recognition by CCD-specific IgE (Mari, 2002). Ana c 2, that is the pineapple protease bromelain, is a well-known example of a glycoprotein detecting IgE antibodies specifically recognizing CCD. Ana c 2 seems to be a very rare cause of allergic symptoms, but nevertheless it is frequently tested positive in serum-based IgE antibody assays. In fact, positive results on Ana c 2 are obtained every time a serum of a subject producing CCD-specific