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Precision medicine in cow's milk allergy: proteomics perspectives from allergens to patients

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ABSTRACT

Cow's milk allergy (CMA) is one of the most common food allergies, especially during childhood. CMA is an immunological mediated adverse reaction to one or more cow's milk proteins, which are normally harmless to a non-allergic individual, as the result of a failure of oral tolerance. To make a correct diagnosis of CMA and a proper treatment is critical in clinical practice. Application of proteomics along with new bio-informatics tools in the field of food allergy is one of the hot topics presented in recent years. In the present review, we focus on recent applications of proteomics to the field of cow's milk allergy, from allergens quantification to the diagnosis, treatment and prognosis. Furthermore, we also shed ~~a~~ light on potential future directions and developments, that are parts of personalized medicine but also of the One Health approach.

Significance: The field of food allergies is becoming a milestone in public health. Food allergies, in fact, can cause life-threatening reactions and profoundly influence the quality of life. Precise, fast and reliable diagnosis of food allergies, and in particular milk allergies is essential to avoid severe allergic reactions and also to prevent dangerous and eventually unnecessary dietary restrictions; but this can be difficult also due to a complex interaction of genetic background, environment, and microbiota. In this sense, proteomics represents steps toward researching food and milk allergy integrated with the clinic to improve pathophysiology, diagnosis, therapy, and prognosis.

1. Introduction

Cow's milk allergy (CMA) is one of the most common food allergies, peaking during childhood, with food challenge prevalence estimated in the range between 0.5 and 3% in Europe, with a decreased rate from northern to southern [1]. CMA is an immunological mediated adverse reaction to one or more cow's milk proteins, which are normally harmless to a non-allergic individual [2], as the result of a failure of oral tolerance. Oral tolerance is the process by which the immune system promotes mucosal and systemic non-responsiveness to orally administered antigens [3].

Although the natural history of milk allergy usually has a positive prognosis, with the majority of children showing resolution during childhood, recent epidemiological data suggest slower rates of resolution and a higher rate of children with persistence of the disease into adolescence and even adulthood [4–6].

Therefore, making a correct diagnosis of CMA and an adequate treatment is crucial in clinical practice. An improper diagnosis of CMA can lead to unnecessary exclusion diets that are not without nutritional risks [7,8].

Currently, the diagnostic work-up of CMA allergy is based on a detailed clinical history and physical examination followed by in vitro or in vivo tests to detect immunoreactivity against some specific allergens [9,10]. The proteins most frequently recognized by IgE in cow's milk are α-lactalbumin (also called Bos d 4), β-lactoglobulin (Bos d 5) and casein (Bos d 8) [11]. However, all milk proteins appear to be potential allergens, even those that are present in trace amounts (i.e. lactoferrin, BSA, immunoglobulins) that cannot be detected by conventional in vitro tests, mainly because of the strong signals of more abundant immune-reactive proteins in the sample. However, a major problem of CMA is the fact that the human IgE response to CMP is characterized by a great variability as an allergic individual may react

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to each one to different epitopes. Therefore, sensitization to several proteins occurs in the majority of patients with CMA, with a great variability of the IgE response in specificity and intensity. The oral food challenge is still considered the gold standard for the diagnosis of food allergies [12], to provide a definitive diagnosis and to prevent the patients from unnecessary and potential harmful diets [13]. However, this test is very time consuming, costly, can only be performed under medical guidance in tertiary centers and bears the risk of inducing severe anaphylactic reactions [14–16]. For this reason, the need to perform oral challenge for diagnosis of CMA in clinical practice is debatable [17].

The science of proteomics concerns the global scale study of proteins component and their interactions of an organism, tissue, cell, biological fluid, in a particular moment and under given conditions.

The potential of proteomics technologies in food allergies are so wide that we are now at the beginning of the new era in allergies [18]. In the field of food and nutrition research this revolutionary approach induced to coin the term *foodomics* as a discipline that studies the food and nutrition domains through the application of advanced omics technologies to improve consumers' well-being, health and knowledge [19–21]. Moreover, these approaches combined to new bio-informatics tools in the field of food allergy represents new challenge for scientific community [22].

In this context, proteomics provides useful information for the identification and structural characterization of allergens through the application of nanotechnologies, enabling the design of proteins microarrays for improved molecular diagnosis. Also, immune epitopes deriving from allergens can be quantitatively investigated applying sophisticated proteomic tools, like targeted mass spectrometry (MS),that is one of the most important techniques in proteomics in order to identify, confirm and characterize proteins; MS can also provide the identity of cow's milk allergens in a complex matrix in which all the proteins are not expressed at the same level, with the most abundant proteins signal covering the low level ones [23]. Proteomic approaches also permit to distinguish different clinical phenotypes of CMA, according to the in vitro sensitization profile [24] and further for searching prognostic biomarkers related to oral food challenge response [25]. Last but not least, the availability of well identified allergen components may lead to the development of hypoallergenic molecules, containing T cell epitopes but lacking IgE epitopes, that should be employed for oral immunotherapy [26,27].

In the present review, we focus on recent applications of proteomics to the field of cow's milk allergy, from allergens quantification to the diagnosis, treatment and prognosis. Furthermore, we also shed light on potential future directions and developments, that are parts of the precision medicine.

2. Cow's milk allergens and peptides

2.1. Milk allergens identification and characterization

Cow's milk (CM) contains nearly 200 different proteins, but only some of them are known to be allergenic [28]. The main cow's milk allergens are caseins, β-lactoglobulin, and α-lactalbumin [29]. Most proteins in milk, including the allergens, are glycoproteins [30,31]. Through acidification at pH 4.6, or ultracentrifugation, these milk proteins could be distinguish in two main fractions: the caseins, that are located in micellar complexes conferring its milky appearance [32,33], which account for 80%, and the whey proteins that represent 20% of the total bovine milk proteins. The casein fraction (Bos d8) includes the following four isoforms in different percentages: α-S1-casein (Bos d9, 32%), α-S2-casein (Bos d10,10%), β-casein (Bos d11, 28%) and κ-casein (Bos d 12,10%). The whey proteins consists of α-lactalbumin (Bos d 4), β-lactoglobulin (Bos d 5), immunoglobulins (Bos d7), bovine serum albumin (BSA, Bos d6) and traces of lactoferrin (Bos d lactoferrin) (Table 1) [34].

Although the most frequently recognized allergens in patients with CMA are caseins (especially α-S1-casein), β-lactoglobulin (β-LG) and α-lactalbumin, no single allergen accounts for a major part of allergenicity and approximately 75% of patients with CMA presents sensitization to several proteins [35]. Most of the traditional tests are able to evaluate specific IgE (sIgE) against only few milk allergens (casein, β-lactoglobulin and α-lactalbumin); moreover, some patients with acute signs and symptoms of CMA do not present high levels of sIgE against the proteins evaluated. In this case the diagnosis of CMA could be established with oral food challenge, the gold standard test for food allergy diagnosis. The presence of proteins in very small amount but with high immunoreactivity makes necessary the application of high sensitivity methods.

Classically, in these biological fluids it can be easily perform the bottom up or the top down proteomics [18]. The bottom up approach, includes two dimensional electrophoresis 2DE and MS method for large-scale analysis of highly complex samples. It uses one or more proteases to digest the proteins into peptides for subsequent MS and MS/MS analyses, as reported in Fig. 1. On the contrary, the "top-down" strategy, that is only MS-based approach, analyzes intact proteins without prior digestion, providing information on intact protein mass and its amino acid sequence. In summary, the bottom up and top down methods constitute complementary approaches, where the bottom up method is generally used for routine identification of proteins in complex mixture, as for identification of food allergens, and post translational modification [18,36]. In particular, proteomics can contribute in two ways to determine food allergens: a gel-based approach, that includes 2D electrophoresis, 2D immunoblotting and MS approach for protein spot identification, or a gel-free approach, characterized by a high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) approach and IgE-binding assay of the trypsined proteome [37].

Several studies in the last years have been conducted through these new techniques with the purpose of a better characterization of milk allergens [38]. Immunological methods combined with protein identification by mass spectrometry lead to detect lactoperoxidase and protein FAM13A, two novel allergenic molecules in bovine milk never reported before [39]. The high sensitivity reached from proteomic analysis applied to human milk has been recently demonstrated [40,41].

Through immunoaffinity capillary electrophoresis coupled to MALDI-TOF mass spectrometry for whey milk analysis, Gasilova et al. have been available to identify β-lactoglobulin and α-lactalbumin even in traces [42].

In studies performed through immunoassay-based techniques before the developing of advanced MS techiniques, β-lactoglobulin was recognized as the major allergen secreted in human milk and therefore responsible for cow's milk proteins (CMP) sensitization observed in breastfed infants [43]. On the contrary, in a recent study the analysis of term and preterm colostrums samples with proteomic tools has detected intact bovine α-S1-casein, but not β-lactoglobulin, in both type of colostrums [44].

2.2. Unrevealing hidden allergens and food safety

Food safety include also food allergens (proteins) inspection that is matter of public health as integral part of One Health Approach [45,46]. In fact, food allergens can be considered dangerous as well as foodborne pathogens, and there is an increasing need to assess food safety faster and on large scale. Accordingly, absolute quantification of allergens by proteomic approaches lead to assess the risk of allergen levels the allergic consumer will be exposed [47]. In turn, this helps the food industry to produce safer foods for the allergic consumer, both traditional as well as novel foods. An additional field in which proteomic offers a great contribution is the discover of hidden allergens in matrix, as more allergic patients react also to traces of allergens