

Editorial

Has *in vitro* allergenicity testing any clinical relevance?

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Cow's milk proteins involved in milk allergy are numerous and heterogeneous, with very few structural or functional common features (1). The quantitative evaluation of the allergenicity of food proteins and the clinical tolerance towards antigens are problems food industry and clinicians have to face. Docena and coworkers reported on the residual antigenicity and allergenicity of cow's milk substitutes evaluated with *in vitro* tests (2). As could be expected, the authors found the lowest residual allergenicity in an amino acid-based product and the highest in native cow's milk (2). Peptides as short as 12–14 amino acids residues may account for a significant part of the allergenicity of cow's milk molecules (1). Niggeman et al. (3) described that skin-prick tests (SPT) and radioallergosorbent test (RAST) inhibition are also suitable methods for determining the residual allergenicity of hydrolyzed infant formulae, while the determination of protein content using the applied modified. The Lowry method was found to be less useful. Indeed, Docena et al. also used the Lowry method, and report with the amino acid-based formula results in the range of native cow's milk (5.25 and 5.1 mg/ml, respectively), while the amount in partial and extensive hydrolysates is much lower (2). However, the authors did not comment on the utility of the Lowry method.

As said above, SPTs were used by Niggeman et al. (3). However, peptides of > 1400 Da may be needed for skin reactivity (4), which are absent in extensive hydrolysates. Extensive hydrolysates do not only show immunoglobulin (Ig)E-antibody binding *in vitro*, some

children with cow's milk allergy react clinically to extensive hydrolysates (5). These children do well on an amino-acid-based formula (Neocate, SHS, UK). However, the majority of allergic children tolerate extensive hydrolysates (5). In a comparative study, 19 and 15% of children had a positive SPT to an extensive hydrolysate, but clinically tolerated the extensive hydrolysate in 97 and 94%, respectively (6). The '100% diagnostic SPT levels' avoids up to 23% of double-blind placebo-controlled food challenges that would have been necessary according to the results of *in vitro* tests (7). Similar findings for IgE-antibody levels have been reported by Sampson et al. (8).

Treatment and prevention of cow's milk allergy constitute different clinical conditions. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition and the European Society of Paediatric Allergy and Clinical Immunology (ESPGHAN/ESPACI) consensus paper states that preventive intervention is recommended in nonbreast-fed infants with at least one parent or sibling that is clearly atopic (9). In the recent EAACI Position Paper atopy is defined as 'a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, or eczema/dermatitis' (10). In fact, the target population for preventive intervention is still a matter of debate. Genetic studies suggested that infants born in atopic families are at increased risk for developing IgE-mediated allergies. However, it is not clearly shown that infants from atopic families also

have an increased risk for cow's milk allergy, although cow's milk allergy belongs to the spectrum of classical IgE-mediated disorders. A prospective intervention study in a large cohort of unselected infants resulted in an increased duration and incidence of exclusive breast feeding (11). If this increased incidence and duration of breast feeding is confirmed in other trials, it may be a good reason to recommend dietary intervention in all infants, independent of the presence or absence of any effect on the incidence of cow's milk allergy. It is obvious that the residual allergenicity in partial hydrolysates is too high to administer this kind of formula in children with cow's milk allergy (6). As a consequence, the introduction in the abstract of the paper of Docena et al. is somehow misleading, because the authors state that their 'study aimed to investigate the presence of residual allergenic cow's milk proteins in some milk substitutes employed in the treatment of cow's milk allergy' (2). Partial hydrolysates are contraindicated in the treatment of cow's milk allergy, as listed on their label. Equally, Giampietro et al. (6) correctly wrote that the American Academy of Pediatrics, the ESPGHAN and ESPACI, endorse the statement that partial hydrolysates should not be used in therapeutic conditions. Yet, Giampietro et al. challenged cow's milk allergic children with a partial hydrolysate (6). Docena et al. state that 'partial hydrolysates were developed to overcome problems in taste, smell, osmolarity and cost of extensive hydrolysates' (2). This is not exactly the case. Partial hydrolysates were developed because they may be more effective in the prevention of cow's milk allergy than extensive hydrolysates, because they may induce a better tolerance than extensive hydrolysates, as suggested by animal studies (12). However, infants cannot be compared to mice or rats. Whether 'a better tolerance induced by partial hydrolysates' is true or not, is still unclear. Clinical trials comparing extensive and partial hydrolysates are inconclusive, or are in favor of extensive hydrolysates, but without demonstrating a statistical and clinical significant benefit (13–14, von Berg A, personal communication). Thus, whether extensive or partial hydrolysates should be preferred in prevention is still a matter of debate.

Results of *in vitro* tests cannot be extrapolated to the clinical situation. The clinical relevance of 'special' *in vitro* tests as applied by Docena et al. gives a good example. Indeed, some of the test products (Nan-HA and Beba-HA) are 'more or less' identical. However, according to the data of Docena et al. Nan-HA scores very poorly and Beba-HA scores well. Equally, Beba-HA2 scores as good as the amino-acid formula (Neocate), although the degree of hydrolysis in Beba-HA2, Beba-HA1 and Nan-HA1 is similar. In principle, Nan-HA and Beba-HA are the same products com-

mercialized under different names. Both products are produced in the same factory in Germany. It is obvious that very sensitive tests may detect differences between products if they are produced at a different moment. Indeed, factories have release criteria, and all products that fulfil these safety criteria are commercialized. However, this can not explain the very large differences reported between Nan-HA and Beba-HA. Moreover, *in vitro* tests do not consider the 'real life' situation in which many variables such as food preparation, environmental factors such as gastro-intestinal tract flora, development of oral tolerance, etc. may finally determine the presence or absence of a clinical reaction. The capacity of a protein to provoke an allergic reaction depends on multiple factors, including the stability to digestion and the interaction with the intestinal environment. In addition to the possible reduction in allergenicity by technological treatments such as heat and enzymatic hydrolysis, the complex interactions existing between the antigens, the intestinal epithelium and the underlying immune system, as well as the individual susceptibility to the sensitizing epitopes, have to be taken into account. The intestinal cells are able to take up and process proteins, and possibly to present them directly to mucosal lymphocytes (15). The difficulty in measuring the residual clinically relevant allergenicity of hypoallergenic formulas is partly owing to the physiology of the gastrointestinal tract, because an intricate network of interactions between enterocytes and immune cells governs the development of the immune response to food antigens (15). Brown Norway rats can be sensitized orally to various allergenic foods tested, resulting in significant antigen-specific IgE responses (16). The specificities of induced antibody responses in Brown Norway rats resemble those found in food-allergic patients (17). In 'at risk' patients oral tolerance can be induced by means of tolerogenic cow's milk peptides or hydrolysate (18). But, none of the hydrolyzed formulas is nonallergenic (19). However, the amino acid-based formula is.

In conclusion, it is highly probable that the linking of 'in vitro testing' for residual allergenicity, i.e., IgE-antibody binding properties, and its 'clinical impact' is very hazardous. At the best, *in vitro* testing confirms that the longer the peptide chain is, the higher the allergenicity. Docena et al. also show that allergenicity may be species-specific. But, this does not mean that raw mare's milk may be considered as an alternative for mother's milk- or cow's milk-based artificial formula. Any artificial formula given to infants should provide optimal nutrition, which is of much greater clinical relevance than allergenicity. Raw milk from any other species than humans is not adapted for infant feeding, and can thus not be recommended to be given to infants.

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