# Food Allergies: Novel Mechanisms and Therapeutic Perspectives

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# Chapter 14

# Food Allergies: Novel Mechanisms and Therapeutic Perspectives

**Margherita Di Costanzo, Lorella Paparo, Linda Cosenza, Carmen Di Scala, Rita Nocerino, Rosita Aitoro, and Roberto Berni Canani**

## Abstract

Childhood food allergy (FA) rates have rapidly increased with significant direct medical costs for the health care system and even larger costs for the families with a food-allergic child. The possible causes of food allergy become the target of intense scrutiny in recent years. Increasing evidence underline the importance in early life of gut microbiome in the development of allergic diseases. There are a range of factors in the modern environment that may be associated with changes to both the gut microbiome and risk of FA, such as mode of delivery, antibiotic exposure, infant feeding practices, farming environment, and country of origin. Knowledge of the relationship between early life gut microbiome and allergic diseases may facilitate development of novel preventive and treatment strategies. Based on our current knowledge, there are no currently available approved therapies for food allergy. More studies are needed to evaluate the safety and efficacy of allergen-specific and allergen-nonspecific approaches, as well as combination approaches.

**Key words** Immunotherapy, Probiotics, Intestinal microflora, Immune system, Tolerance acquisition

## 1 Introduction

Food allergy (FA) is a major health issue in Western countries with a substantial effect on quality of life of both patients and their relatives. On the basis of numerous studies, food allergy likely affects nearly 5 % of adults and 8 % of children, with growing evidence of an increase in prevalence [1]. Although any food can provoke a reaction, relatively few foods are responsible for the vast majority of significant food induced allergic reactions: cow's milk (2.2 %), peanuts (1.8 %), and tree nuts (1.7 %) are the most common allergens in children, and shellfish (1.9 %), fruits (1.6 %), and vegetables (1.3 %) are the most common allergens in adults. Recent publications focusing on peanut allergy indicated increases with a doubling (UK) or tripling (USA) in diagnoses [2, 3]. In general, childhood FA to milk, egg, wheat, or soy typically resolves during childhood, whereas allergies to peanut, tree nuts, fish, and shellfish

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are persistent. However during the last decade, a changing pattern in FA was observed with an increased prevalence, severity of clinical manifestations, and risk of persistence until later ages in Western countries [4]. Over the last 20 years rates of potentially life-threatening reactions to food (anaphylaxis) have steadily risen in the developed world [5]. For these reasons, there is a strong need for an expansion of pre clinical research to improve understanding of the mechanisms and to develop novel effective strategies to prevent and treat FA.

## 2 New Insights into the Pathogenesis of Food Allergy

There is a complex interplay of environmental influence and genetics that underlie the immunopathogenesis of food allergy and the manifestations of various food-induced allergic disorders. The gut microbiota is emerging as a crucial "internal" environmental exposure [6]. We briefly summarize what are the evidences that demonstrate an association between microbial exposure in early life and the development of food allergy.

Microbial gut colonization begins after birth and this process is affected by the newborn infant's gestational age, mode of delivery and first feeding strategies. The colonizing bacteria originate mainly from the mother's gut and vaginal tract [7]. After delivery, breast feeding continues to enhance the original inoculum by the introduction of specific lactic acid bacteria, Bifidobacteria, and other bacteria from the mother's skin. These bacteria set the basis for gut microbiota development and modulation. An imbalance in the compositional configuration of the gut microbiota, dysbiosis, alters the host-microbiota homeostasis, which is a requisite for the development and function of immune cells in the gut associated lymphoid tissue. The importance of this reciprocal regulation of the microbiota and immune system culminates in early infancy, when the balance between homeostasis and inflammation programs later disease risk. In particular, early exposure to commensal bacteria plays a crucial role in Th1/Th2 polarization and proper immune regulatory mechanisms. Germ free animals do not develop oral tolerance and maintained a Th2 type immune response to orally administered ovalbumin. This could be corrected by the reconstitution of the microbiota at the neonatal stages, but not any reconstitution implemented at a later ages [8]. These findings documented a decisive role of the gut microbiota for the acquisition of food oral tolerance in early life. Exposure to a normal intestinal microflora in early life allows for a change in the lymphocyte Th1/lymphocyte Th2 balance, favoring a Th1 cell response [9], while an imbalance in the compositional configuration of the gut microbiota, dysbiosis, alters the host-microbiota homeostasis, producing a shift of the Th1/Th2 cytokine balance toward a Th2

response and a consequent activation of Th2 cytokines with an increased production of immunoglobulin E [10]. Imbalance in intestinal microbiota composition has been documented in patients with food allergy [11]. It was recently found that clostridia strains promote the development of regulatory T cells in the intestine, and when a mix of human clostridia strains were administered to mice, they could suppress the development of food allergy [12, 13]. Recently, a new link between dysbiosis and food allergy development has been provided. Maternal use of antibiotics before and during pregnancy was associated with an increased risk of cow's milk allergy in the offspring and the risk of cow's milk allergy increased with increasing number of child's antibiotics used from birth to diagnosis [14]. In a recent review Marrs et al. conducted a systematic review to test the hypothesis that microbial exposure can modulate the risk of developing FA and concluded that factors influencing microbial exposure, such as mode of delivery, rural animal exposure, diet, childhood infections, immunizations, and antibiotic use, may be partly responsible for rising FA burden, but further prospective studies using double-blind placebo controlled food challenges as an outcome are required [6].

## 3 Allergen-Specific and Non-allergen-specific Therapies

Based on our current knowledge of the immune basis of food allergy, therapeutic strategies have focused on reducing levels of allergen-specific IgE, enhancing levels of allergen-specific IgG or IgA, suppressing Th2 effector cells, or enhancing regulatory T cells through a variety of allergen-specific and allergen non-specific strategies [15].

### *3.1 Allergen-Specific Therapies*

In the past 10 years allergen immunotherapy by the oral, sublingual, or epicutaneous routes has been the subject of intense research focus. Results are promising when desensitization, defined as protection from food-induced reactions while receiving therapy, is used as a primary outcome [16–18]. However, there is a lack of clarity about safety and long-term efficacy of the treatment. Adverse reactions to oral immunotherapy are not uncommon, and a significant number of subjects experience adverse reactions of sufficient severity or persistence to prevent continuation of immunotherapy. The most successful trials report that at least half of patients who begin immunotherapy do not achieve successful long term tolerance [19, 20]. Preclinical research on food allergy immunotherapy safety has primarily focused on modifications to allergen structure to reduce IgE binding. Allergens can be modified through heating, which denatures the proteins and destroys conformational epitopes; digestion, which forms peptides that are too short to cross-link IgE but maintain T cell epitopes would have the capacity to generate

T cell-mediated immunomodulation; and chemical modification, for example glycosylation of allergens can significantly modify their immunogenicity and allergenicity [15].

### 3.2 Modified Allergens and Adjuvants for Allergen Immunotherapy

Some allergens can be modified simply through heating. Heating denatures the proteins and destroys conformational epitopes, and there are also matrix effects that influence digestion and absorption of the allergens. Milk- or egg-allergic children enrolled in intervention studies in which they incorporated extensively heated milk or egg into the diet outgrew their unheated egg or milk allergy more quickly than a control group that received standard of care [21, 22], and this inclusion of milk or egg was associated with changes in immune parameters consistent with an immunotherapeutic response (elevation in IgG4, decreases in allergen-specific IgE). Heating to reduce allergenicity is applicable to egg or milk, but not to antigens such as peanut where high heat increases allergenicity rather than reducing it. Allergens can be also modified through digestion, which forms peptides that are too short to cross-link IgE but maintain T cell epitopes would have the capacity to generate T cell-mediated immunomodulation. Immuno-dominant peptides in the peanut allergens Ara h 1 [23] and Ara h 2 [24] have recently been identified with the goal of developing peptide immunotherapy. In addition to digestion and heating, allergens can be modified by chemical modification. Glycosylation of allergens can significantly modify their immunogenicity and allergenicity. Carbohydrate structures can both promote and suppress allergenicity. There is evidence that exposure of some allergens to high heat can enhance allergenicity through glycation, which allows for recognition of the allergens by pattern recognition receptors on antigen-presenting cells [25, 26]. But glycosylation can also result in enhanced immune tolerance. At preclinical level, research on improvements in efficacy of food allergy immunotherapy is focused on adjuvant optimization. Adjuvants that amplify either a Th1 response or a regulatory response may be necessary to sufficiently suppress the Th2-skewed immunity that drives the allergic response to foods. Many of these adjuvants are of microbial origin and range from whole heat-killed bacteria to co-administered purified microbial products to fusion proteins incorporating allergen and adjuvant in one. By binding to innate pattern recognition receptors on antigen-presenting cells, these adjuvants are thought to drive the T cell response away from a Th2 response. Adjuvants not only modify the nature of the immune response, but amplify the response such that significantly lower doses of allergen may be sufficient for an immunotherapeutic effect. The immune basis of tolerance induced by allergen immunotherapy for food allergy is still the subject of intensive research; immunotherapy is associated with elevations in allergen-specific IgG4 and IgA, and reductions in diversity of epitopes recognized by allergen-specific IgE, skin prick test wheal

size, allergen-induced basophil activation, and allergen-induced Th2 cytokine production [27–29]. These parameters are associated with immunotherapy, but so far there have been no biomarkers described that successfully predict tolerance versus desensitization in response to immunotherapy.

### 3.3 Allergen-Nonspecific Therapies

Therapies that are not allergen specific are especially attractive because many patients have multiple food allergies and allergen immunotherapy with specialized allergen adjuvant constructs may be of limited value in these patients. In this field, there is a significant interest in probiotics and the possibility of manipulating the microbiome for therapeutic purposes. Recently, we demonstrated that treatment of cow's milk allergy (CMA) infants with an extensively hydrolyzed casein formula (eHCF) supplemented with the probiotic *Lactobacillus rhamnosus* GG (LGG) accelerates oral tolerance acquisition to cow's milk [30, 31]. Subsequently, we tested the hypothesis that eHCF plus LGG induced effect on oral tolerance thanks to an influence of this dietary intervention on the composition of the gut microbiota (Fig. 1). High-throughput sequencing technology (16S rRNA-based sequence analysis) was used to compare fecal samples from newly diagnosed CMA infants, collected before and after treatment with eHCF plus LGG, to

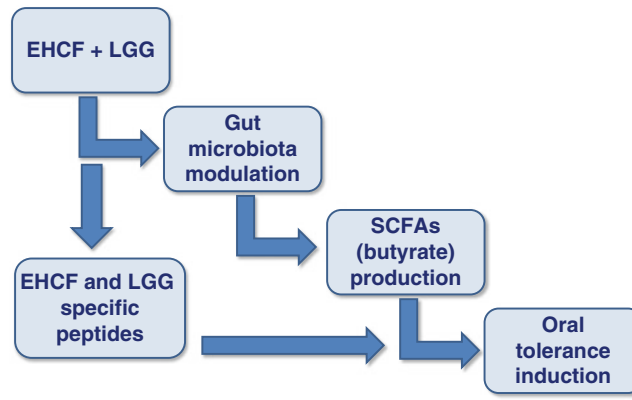


**Fig. 1** A schematic representation of the potential mechanisms of action of eHCF plus LGG in children with cow's milk allergy. Treatment with EHCF plus LGG expanded gut microbiota populations associated with immunoregulatory effects and increased butyrate production at intestinal level. Gut microbiota is a crucial factor for food oral tolerance and it regulates an appropriate balance between immune effectors and regulatory pathways. Short chain fatty acids (SCFAs) such as propionate, acetate and butyrate are gut microbiota-derived bacterial fermentation products that selectively expand Tregs in the large intestine. These SCFAs stimulate the expansion and immune-suppressive properties of Tregs, such as the production of IL-10. Moreover, the specific immunomodulatory effect of eHCF plus LGG may be due to small specific peptides, which are absent in other formulas

those obtained from controls. Treatment with eHCF plus LGG expanded gut microbiota populations associated with immunoregulatory effects. Otherwise healthy infants with CMA were given eHCFs ($n=55$), eHCF with LGG ($n=71$), hydrolyzed rice formula ($n=46$), soy formula ($n=55$), or amino acid-based formula ($n=33$), and oral food challenges were performed after 12 months to assess acquisition of tolerance. The rate of tolerance after 12 months was significantly higher ($p<0.05$) in the groups receiving eHCF (43.6 %) or eHCF plus LGG (78.9 %) compared with the other groups: hydrolyzed rice formula (32.6 %), soy formula (23.6 %), and amino acid-based formula (18.2 %). Our in vitro and in vivo data suggest that eHCF containing LGG promotes oral tolerance through a combination of different mechanisms. These findings suggest a potential innovative therapeutic approach for children affected by FA.

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