Cracking the egg: immunological and molecular analysis of egg allergens

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Abstract

Egg allergy is a wide spread condition which affects mainly children. Of all the other food allergies, egg allergy is the second most common one and has a high prevalence in developed countries. Egg allergy is caused by antigens found within the egg white and the egg yolk. Four egg white allergens have been identified and extensively studied over the past two decades, and the two identified egg yolk allergens need further studies to confirm their allergenicity. These allergens may not be the only allergens in the egg since there are novel egg proteins discovered via recent research. Currently there is no definite cure for egg allergy, with strict avoidance being the primary method of management of the condition. However strict avoidance is extremely difficult due the presence of traces of eggs in a range of food products, pharmaceuticals and vaccines. Due to the high prevalence of egg allergy and the absence of an effective treatment regime, it is important to conduct research on the egg allergens and the underlying mechanisms of egg allergy. This presentation briefly highlights the mechanisms of allergic diseases, egg allergy, egg allergens and current treatment methods.

Keywords

Allergen, antigen, egg allergy, hypersensitivity, immunoglobulin E (IgE)
Allergy

The immune system of the human body reacts to harmless environment substances, such as grass pollen and eggs, by eliciting an immunoglobulin G (IgG) driven immune response, which is harmless to the individual. However, some individuals react to these innocuous environmental substances via an immunoglobulin E (IgE) driven pathway, which causes harm to the individual, a condition termed allergy and/or type I hypersensitivity (1). In allergy, the usually harmless environment substances are known as allergens and they are antigens derived from a range of sources; from food to pharmaceutical products. The allergic reaction takes place in two reaction phases; primary response and the secondary response. The primary response refers to the binding of the allergen to antigen presenting cells (APCs) and the subsequent excessive production of allergen specific IgE. These IgE then bind to high affinity receptors found on mast cells and basophils (2-4). The secondary response refers to the binding of the same allergen to multiple IgE, already bound to mast cells and basophils. Cross linking of these IgE causes a signal cascade resulting in degranulation of mast cells and basophils, releasing chemical mediators that elicit clinical symptoms of allergy. These symptoms include, but not limited to, itching, sneezing, rashes and life threatening conditions such as anaphylaxis (1, 5-7).

Diagnosis of allergy

Scientific advances in delineation of antigen presentation and molecular signalling have provided insights into the underlying principles of the immune response to allergens and the subsequent synthesis of IgE which leads to the clinical symptoms of allergy. This framework has assisted in developing the two widely used diagnostic approaches of allergy; skin tests and blood tests. A skin test involves the introduction of a small amount of a known concentrated allergen into the epidermis (8). If the individual is allergic to the introduced substance, a blister or a wheal (redness) takes place. The severity of the reaction is determined by measuring the diameter of the affected area. A blood test involves quantification of IgE both as total immunoglobulin concentration and as allergy-specific antibody concentration in the patient’s serum. The IgE levels are quantified mainly by using
radioallergosorbent tests (RAST) in which radioisotope-labelled IgE binding antibodies are used to measure serum IgE levels(8).

**Treatment of allergy**

The most common approach of allergy treatment focuses on allergen avoidance and pharmacotherapy to neutralize allergic symptoms. Pharmacotherapy exploits antagonistic drugs such as antihistamines, antileukotrienes, corticosteroids, cromolyn, methylxanthines, β-agonists, muscarinic antagonists and mast cell stabilisers to block allergic mediators or to alleviate the degranulation process (8, 9). However, such therapeutics does not yield curative results due to their inability to inhibit IgE production. Furthermore, antagonistic drugs are capable of promoting immunosuppression or non-immunological effects (10).

Allergic desensitization through administration of appropriate concentrations of allergens has been conventionally used to improve immunological tolerance in allergic individuals. Allergen-specific immunotherapy (SIT) is a desensitizing therapy that has been used practically for a century and represents the only remedial and allergen-specific treatment method. Allergen-SIT essentially involves weekly vaccination (subcutaneous) of increased doses of specific allergens into the patient with the aim of modifying Th2 lymphocytes into Th1 lymphocytes and therefore inducing Immunoglobulin G (IgG) production instead of IgE. This method has been recognized as an effective treatment for rhinitis and asthma (10).

Sublingual immunotherapy (SLIT) is another form of SIT, which involves administration of allergen doses via the sublingual mucosa. An analysis of multiple SLIT studies has confirmed the efficacy of SLIT on reducing rhinitis symptoms and the necessity of other medications (11). However, administration of increased amounts of allergens may cause excessive production of IgE and lead to therapy-induced anaphylaxis.

**Food allergy and the intestinal immune system**

Food allergies arise through the involvement of the intestinal immune system, which is the largest, most complex and one of the most important parts of the immune system. The
intestine plays a major role by distinguishing between harmless antigens and harmful antigens, pathogens and microorganisms. The intestine is exposed to different types of antigens and microorganisms than any other body part and this highlights the importance of having a powerful immune system to protect this tissue and the rest of the body. Non-allergic individuals demonstrate induction of intestinal local and systemic immunological tolerance to non-pathogenic antigens, a phenomenon known as oral tolerance. Antigens entering the intestine initially encounter microfold cells (M-cells) prior to being presented to APCs for processing. M-cells are a type of enterocytes found on the epithelium and they lack the mucus layer. APCs then present the antigen to Th-0 cells. In areas such as the lamina propria of the intestine, MHC class II enterocytes act as APCs and present the antigens to Th-0 cells. In both cases, the Th0 cells prime as intolerant cells, which causes B-cells to differentiate into IgA secreting plasma cells(12). A strong physical barrier, presence of antigen altering luminal enzymes, presence of specific regulatory T-cells in the lymphoid tissue of the gut, and the production and secretion of IgA capable of functioning in the hostile gut environment ensures immunosuppression of the intestinal immune system upon contact with an antigen, therefore causing antigen tolerance. Antigen tolerance is essential because eliciting an active immune response to harmless food antigens can be wasteful. Food intolerance or food allergy arises due to defects in one or more of the components responsible for tolerance. As in many other allergies, genetic predisposition to produce IgE causes oral tolerance to fail or break down(13, 14).

Different types of food allergies arise through the interaction between the intestinal immune system and food allergens present in the food that we consume on a daily basis. Allergenic foods include, but not limited to, eggs, peanuts, fish and milk. Antigens (allergens) present in these foods are responsible for IgE mediated hypersensitivity reactions. For example, people allergic to milk react to the milk protein β-lactoglobulin (BLG)(15). Most of these allergens are glycoproteins that can withstand heat, acids and proteases(14), this explains why most allergenic foods remain allergenic even after cooking or processing. A large number of plant and animal proteins responsible for food allergy have been identified and characterized. Majority of the plant food allergens belong to the cupin and prolamin superfamilies, profilin and plant defense proteins. Profilin allergens shows high cross-reactivity to pollen and other food allergens(14). When compared to plant allergens, animal
Egg allergy

Egg allergy is a type I hypersensitivity reaction caused by allergens present in the egg white and the egg yolk. Compared to other allergies, this condition is more common among children, although allergy to the egg yolk can affect adults. Its prevalence as a food allergy is second only to cow’s milk allergy and it is the predominant food allergy among children, mainly in the developed world (17-19). Clinical symptoms of egg allergy include vomiting, itching, rhinitis, conjunctivitis, laryngeal oedema, urticarial and anaphylaxis, just to name a few (20-22). Allergy to egg white is caused by four major proteins present in the egg white; ovomucoid, ovalbumin, ovotransferrin and lysozyme. Egg yolk allergy is caused by the currently identified allergens α-livetin and YGP-42. Allergy to egg may not be limited to these identified allergens, as there are novel egg proteins identified in recent studies (23). The allergenic proteins are exclusively produced by the tubular gland cells in the oviduct of the chicken and the production is known to be induced by estrogen (24). Therefore, it can be suggested that egg allergy is not related to allergy to chicken meat since these proteins are not expressed anywhere else in the chicken. It has been shown that contact with raw eggs predominantly causes allergic reaction, however some patients will react to cooked egg (25), possibly due to the heat resistant properties of some allergens present in the egg. However, digestive enzymes or heat does not always affect the IgE reactivity of allergenic proteins (26).

Of all the allergens in the chicken egg, ovomucoid is the most allergenic protein and functions as a trypsin inhibitor. This 186 amino acid protein is highly heat and proteinase resistant, making it responsible for allergic reactions caused by cooked eggs (27-29). Ovalbumin, although slightly less allergenic, is the most abundant allergen in the chicken egg and deemed to be the second most important allergen in egg allergy, especially due to its abundance of 58% of total egg proteins. This 385 amino acid glycoprotein is known to be
heat resistant, however it is not resistant to digestive enzymes (30-32). Ovotransferrin is the third important allergen in the egg, which has shown to exhibit IgE reactivity in both cooked and raw eggs. Its IgE reactivity is related to it structure, in which unfolding of the protein causes an increase in IgE reactivity and disruption of disulfide bridges lead to a decrease in IgE reactivity (33, 34). Lysozyme is the fourth known allergen in the egg white, which is an anti-microbial protein. Even though lysozyme is less allergenic than the aforementioned egg white allergens, its wide use in food products and pharmaceuticals increases its chances of contact with egg allergic patients (35, 36). The two egg yolk allergens, α-livetin and YGP-42, are poorly studied and yet to be fully elucidated. Of the two, α-livetin is the most allergenic and is known to cause bird-egg syndrome, in which α-livetin present in other birds and other body parts of the chicken predispose individuals to the α-livetin found in chicken egg (37, 38).

Egg allergy is diagnosed via a combination of medical history assessments, skin tests and blood tests. When an incident is reported, the foremost step is to analyse the medical history of the patient. Usually, the patient is advised to follow an elimination diet if the patient is unsure about the exact food that caused the allergic reaction. This is then followed by a skin test or RAST, which is done under medical supervision since egg allergy may cause anaphylaxis (39-41). Currently, there is no treatment for egg allergy. The most effective way of avoiding egg allergy is the dietary avoidance and/or contact with egg allergens. Even this management strategy has difficulties since traces of eggs can be found in a range of food items, pharmaceuticals and vaccines. In addition, complete avoidance of eggs in a child’s diet can be disadvantageous in a nutritional value perspective (42). In the accidental exposure and a subsequent reaction to eggs, self-administered epinephrine (such as Epipen) is the only relief (43).

The prevalence of egg allergy and the absence of effective treatment strategies highlight the importance of egg allergy research. Immunological and molecular analysis of all the egg allergens may provide further insight into mechanisms of pathogenesis of egg allergy. An understanding of the immunological mechanisms of the allergens allows researchers to conduct studies on development of hypoallergenic variants of allergens that can be used in immunotherapy. It is also imperative to further analyse egg white and yolk proteins, including the recently discovered proteins, to scrutinize their potential IgE reactivity. The
use of advanced molecular biology techniques in biomedical science is ever increasing and these can be utilised to tackle egg allergy from the root of the course, such as genetic modifications to change or knock out allergenic genes. Although there is no guarantee of curative results, these options seem promising for better management of egg allergy.
References

Author’s biography

Associate Professor Suphioglu graduated with PhD from the University of Melbourne in 1994 and has over 20 years of research experience (h-index: 24) and an international recognition on the (i) molecular and environmental analysis of pollen, latex and nut allergens, (ii) molecular analysis of phosphoinositide 3-kinase (PI3-K) isoforms in cardiovascular disease and (iii) importance of omega-3 fatty acids and zinc in human neuronal cell survival and epigenetics. Associate Professor Suphioglu has more than 80 publications and several patents. He is currently the Associate Professor of Biomedical Science, Course Director of Bachelor of Biomedical Science and Head of the NeuroAllergy Research Laboratory (NARL). (102 words).

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