

IgG₄ and Food Allergy

by
Jay Weiss, Ph.D.
and
Gary Kitos, Ph.D., H.C.L.D



IgG₄ and Food Allergy Introduction

Food allergies are one of the easiest and most difficult diagnoses to ascertain. In anaphylactic reactions immediately following ingestion, the offending food is usually easily surmised. With less severe symptoms and time lags in reactions, the cause can be more difficult to determine. IgE, the mediator of inhalant allergy, only accounts for approximately 20% of food allergic reactions. Therefore non-IgE mediated allergic reactions are most common in food reactive patients and testing only for IgE mediated disease is not a viable strategy. IgG₄ allergen specific antibody tests have been used to help define the cause of allergic reactions for patients with non-IgE mediated food allergy, but IgG₄ serology is not as well studied in this application.

Responses and Effector Roles

Studies of IgG serology have indicated that certain food proteins induce more IgG₄ antibodies than predicted by the IgG₁ response. For example in one study, Calkhoven et al., of unselected children in The Netherlands, egg white, banana, and cow's milk specific IgG₄ were over-expressed when compared to IgG₁ antibody responses in the same children. In these children, peak IgG₄ levels were seen in the 3-5 year old grouping, which was similar to the study of Moroi, which found peak levels at 4 – 5 years of age. In another study of adults with celiac disease and controls with other gastrointestinal diseases, all subjects demonstrated high levels of IgG₄ to cow's milk components. In general, IgG antibodies to food have been shown to be more common and at higher levels in patients with increased intestinal permeability, (e.g. celiac disease, IBS) and oral ulceration than normal patients. Presence of food specific IgG antibodies is an indication of exposure and may suggest compromised intestinal permeability that may be transitory or chronic in nature.

IgG₄ antibodies are induced by long term exposure to antigen as in immunotherapy and can be formed in the development of a specific IgE response. One of the IgE induction pathways includes B-cell switching from IgM to IgG₄ production and potentially to IgE B-cells. IgG₄ antibodies also have been described to bind several cell receptors including those found on basophils. Studies have demonstrated basophil activation with specific anti-IgG₄ monoclonal antibodies in both atopic and normal patients. In pretreatment insulin diabetics, basophil activation by insulin was shown to be caused by IgG₄ auto-antibodies to insulin. Studies with purified dust mite allergen, Der p2, demonstrated that IgG₄ and IgG₁ could enhance basophil histamine release when IgE bound to a non-overlapping epitope. However, in the absence of IgE, neither IgG₁ nor IgG₄ antibodies could activate the basophils when exposed to the same allergen. In other reports heat stable antibodies have been shown to be involved in anaphylactic responses to several food allergens with allergen specific IgG₄ present. In one report, antibody clones were created from an individual receiving immunotherapy. IgG antibodies of each subclass were represented in the clones. Three of the clones were of the IgG₄ subclass of which one enhanced the IgE mediated response to birch allergen while the other two had no effect. In addition a recombinant Fab of the IgG₄ also enhanced the IgE mediated response, indicating that the whole IgG₄ antibody was not necessary for augmenting the IgE reaction. IgG₄ appears to have conflicting roles, mostly protective in immunotherapy and possibly activating in food allergy.

In additional studies, IgG₄ antibodies have been shown to protect beekeepers from

anaphylactic reactions to bee venom. Beekeepers have high levels of IgG₄ specific to bee venom, and their serum passively prevents anaphylaxis in a sensitive individual. Allergen immunotherapy has been shown to induce high levels of specific IgG₄ and is associated with symptom relief⁹. IgG₄ may be utilized to determine if new antigens or different doses are necessary when immunotherapy is unsuccessful. However, the presence of IgG₄ in some food allergic patients did not prevent reactions when individuals were challenged with the offending food¹⁶. One difference between these findings is that food is processed through mucosa while immunotherapy and venom IgG₄ induction is through parenteral exposure. A protective role for IgG₄ is strongly supported for inhalant and venom immunotherapy. However, protection by IgG₄ does not occur in food allergic individuals.

Biochemical Characteristics

IgG₄ does not activate the classical complement cascade and has some unusual biochemical characteristics. It has been shown to exchange half-antibody molecules with other IgG₄ antibodies. Immunoglobulin molecules are comprised of two identical heavy chains with two identical light chains and are arranged with each heavy chain associated with one light chain forming a half molecule. The whole molecule is two identical half molecules held together by disulfide bonds. For IgG₄ molecules it has been shown that whole molecules can trade half molecules and become hybrid antibodies under certain physiological conditions. In fact, it has been demonstrated that IgG₄ antibodies with specificity to two different antigens, bispecific antibodies, can be found in human serum. This helps to explain one of the more unusual characteristics of IgG₄, the inability to precipitate antigen. With bispecific antibodies one would not expect precipitation to occur. IgG₄ antibodies arise after long term exposure and may serve to help prevent inflammatory responses to antigens to which one has chronic exposure because of its ability to exchange half molecules and prevent precipitation.

Mast Cells

As reviewed by Malbec and Daëron mast cells have IgG receptors and are known to be heterogeneous. Mast cells mature differently in various tissues because of local signaling. It has been demonstrated that bone marrow derived mast cells (BMMC) are immature and contain both activating and inhibitory signaling IgG receptors. Serosal mast cells mature from BMMCs mostly due to stem cell factor (SCF) signaling from local tissues. They have been shown to lose the inhibitory IgG receptor mechanism, making them more reactive with IgG antibodies. These same serosal mast cells can be inhibited from IgE activation by specific IgG₄ antibodies and support the protective role of IgG₄. Serosal mast cells are found in the skin, sub-mucosa of the respiratory tract, joint synovium, and the peritoneum. Another mast cell, the mucosal mast cell, matures from BMMCs in response to T-cell cytokine stimuli, including IL-3, and is found in the gastrointestinal tract as well as the mucosa of the respiratory tract. Little is known about how the mucosal mast cells interact with IgG or IgG₄ although there is clearly the potential for both inhibition and activation of mast cell response.

Identification of Allergic Foods

All assays have strengths and weaknesses that need to be considered when applying them in a diagnostic setting. Ideally the interactions of the marker in normal and

pathological physiology are known. In best cases, the marker is associated with the cause of the disease. Clearly IgE antibodies are associated with allergic reactions, but are found in non-allergic individuals. IgG₄ antibodies are not as well correlated with allergic reactions. IgG₄ antibodies are associated with the development of IgE responses as described above and seem to have roles in protection from allergy as well as exacerbating the allergic condition. IgG₄ is closely associated with chronic exposure to an antigen. There may be some relevance to the route of exposure and the effector function of the antibody. Injection therapy and natural animal exposure¹¹ may induce more protective functions and enteric exposure may not. It is also clear that IgG₄ antibodies to foods are not unusual. IgG₄ antibodies to allergen are found in normal non-symptomatic people, however, specific IgE can also be found in normal non-symptomatic individuals.

In one clinical study, patients were orally challenged with food and placebo capsules, subjects were skin tested and tested for specific IgE and IgG₄ antibodies to each food. Twenty-five subjects were selected for the study based on histories suggesting food allergy. Symptoms associated with ingesting food included, rhinorrhea, urticaria, eczema, asthma and diarrhea. Food challenge positive reactions were classified as immediate or delayed and included wheezing, urticaria, edema, rhinorrhea, drop in blood pressure, bronchospasm, flare up of eczema and generalized pruritis. Twenty of the 25 subjects (80%) had positive challenge results. Skin test results were positive in 29% of the positive food challenges. Specific IgE was positive in 63% of the positive challenges, as was IgG₄. Although there was some overlap, when IgE and IgG₄ were used together, 91% of the positive food challenges were detected. Using either serological test alone with skin testing results detected only 71% of the positive food challenges. Adding skin testing results to the combination of both serology results did not improve the detection of positive challenges. This study indicates that IgE and IgG₄ when used together can be a powerful tool for identifying the cause of food allergy.

Conclusion

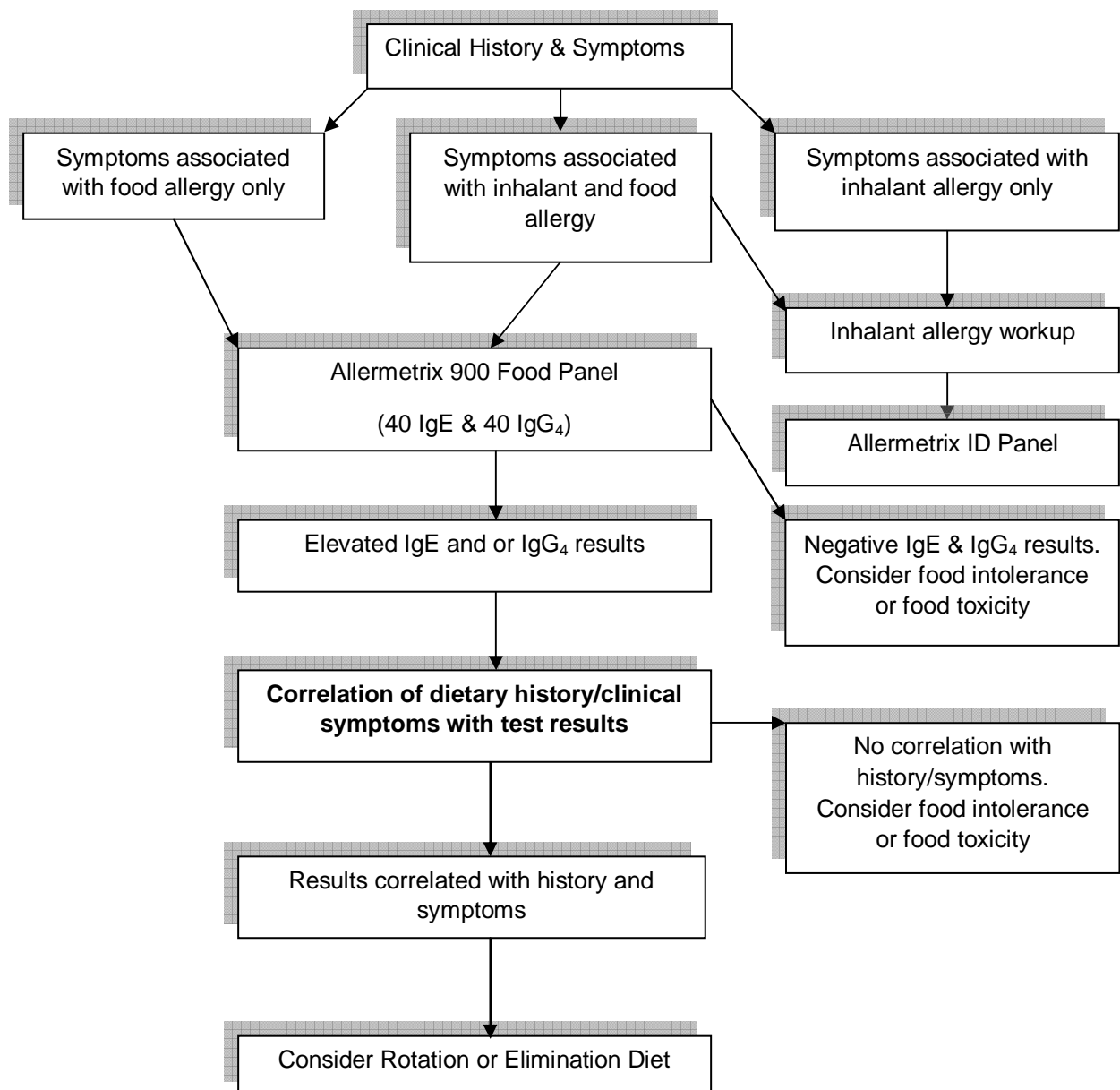
The importance of IgG₄ food specific antibodies has been relegated to an interesting epiphenomenon by some because the cause and effect connection to allergy is much weaker than that of IgE, but it is the nature of laboratory medicine to be empirical. When a marker adds information in specific diagnostic situations it should be used. IgG₄ is a serological marker that should not be forgotten when patients present with symptoms of food allergy. Together with specific IgE, IgG₄ can identify foods that may cause the allergic condition.

Food Allergy Testing Strategy >>>

A Strategy to Identify the Food Allergic Patient

When developing a strategy to identify food allergy, various factors must be taken into effect; dietary history, clinical symptoms, IgE mediated responses, non-IgE mediated responses (IgG or IgG₄), allergen antigenicity, hidden foods and cross-reactivity of foods with airborne pollens. Approximately 90% of food allergy is found in the following foods/food groupings; tree nuts, milk, peanuts, milk, eggs, soy, wheat, fish and shellfish.

Laboratory Algorithm for Food Testing



Allermetrix-Food Panel (#900)

Food allergies are difficult to diagnose and often the allergens causing disease are not obvious. The literature indicates there are a number of foods that are most often identified. Allermetrix has constructed a comprehensive food allergy panel that incorporates data from published clinical studies and our in-house testing results. For the best diagnostic efficiency both IgE and IgG₄ antibodies must be tested to identify the offending allergen(s). Often food allergy is not IgE mediated requiring the need to assay for IgG₄ reactivity (believed to be involved in delayed reactions).

Allermetrix reviewed over **15,000** food tests submitted to the laboratory and identified the most commonly positive allergens. The rate of positive results is highest for IgG₄ milk and egg reactions, but other groups of food also demonstrate high rates of reactivity for both IgE and IgG₄. After analysis of individual allergens, 40 foods were selected for the “900” food panel.

The allergens included in the 900 panel are grouped below:

- **Legumes:** Bean (White/Navy), Pea (Green), Peanut, Soybean
- **Tree nuts:** Almond, Brazil, Cashew, Coconut, Hazelnut, Pecan, Pistachio, Walnut (English)
- **Fish:** Specific fish only if indicated on diet history
- **Shellfish:** Specific shellfish only if indicated on diet history
- **Dairy:** Cow's Milk
- **Animal:** Beef, Egg White, Egg Yolk, Pork
- **Fruits:** Apple, Banana, Cantaloupe/Muskmelon, Grape/Raisin, Lemon, Orange, Pineapple, Tomato, Watermelon
- **Seeds/Spices:** Mustard, Sesame Seed, Sunflower Seed
- **Grains:** Barley, Gluten, Oat, Rye, Wheat,
- **Vegetables:** Celery, Garlic, Onion, , Potato, Pepper (Bell)
- **Yeast:** Yeast/Baker's

*-Often times egg and milk IgG₄ results are elevated in normal patients.

Note: Fish and shellfish are not included in the panel and should be tested as indicated from the diet history.

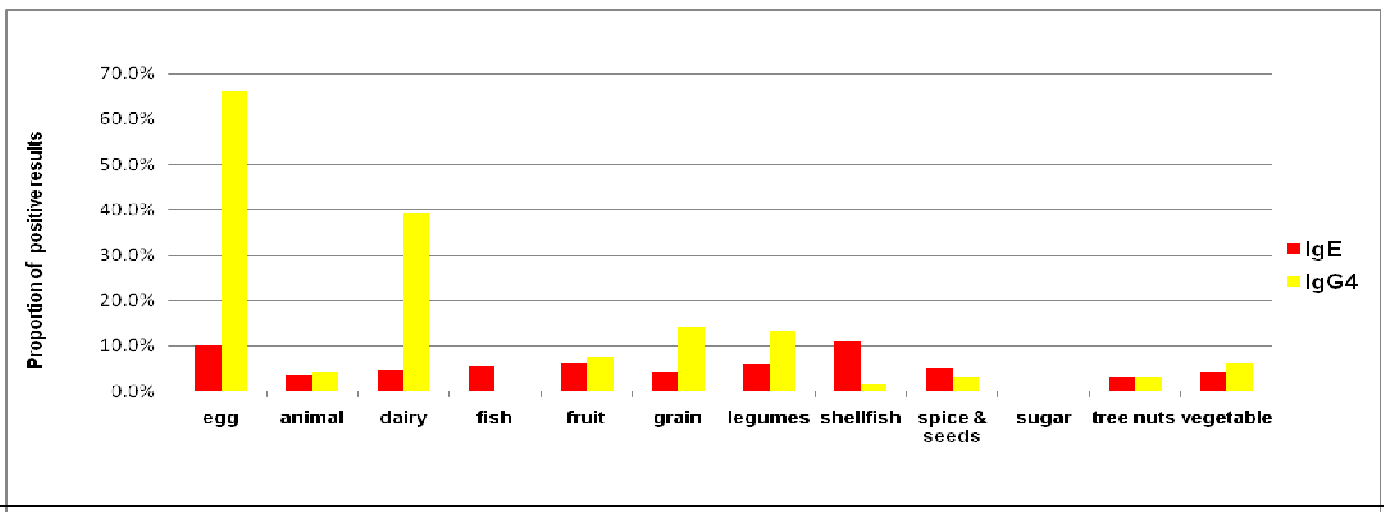
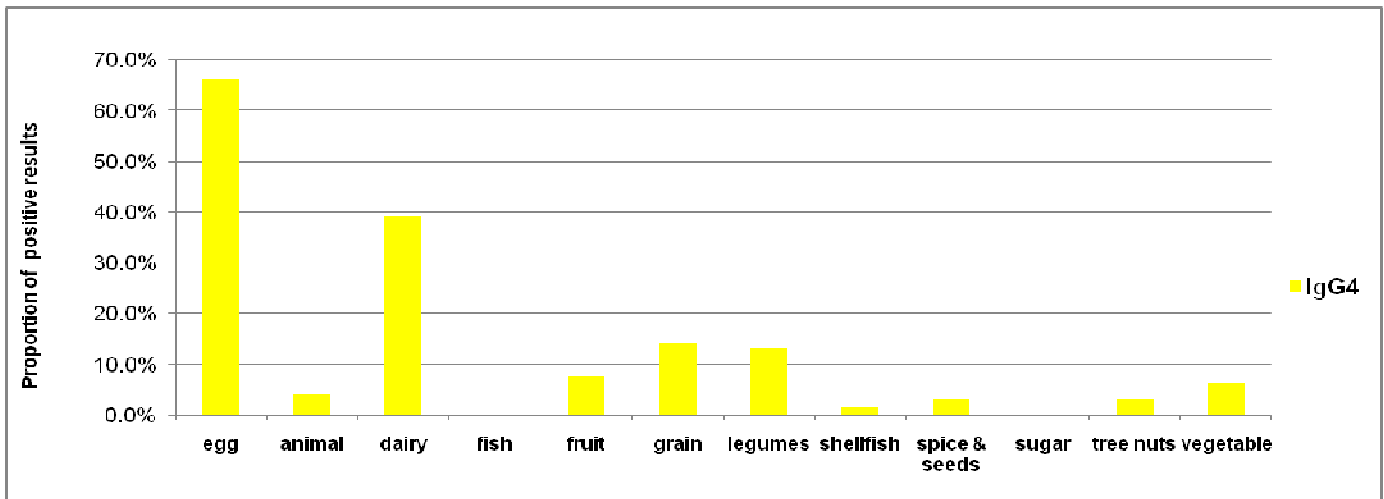
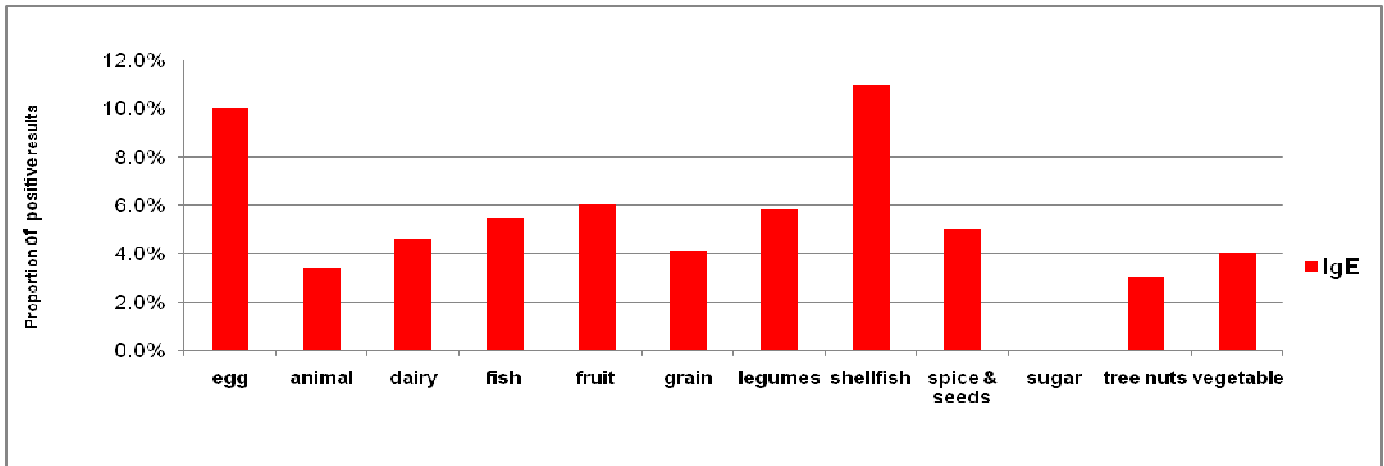
Improving Allergy Outcomes Through “Applied Science”

By incorporating the best information available in the literature, the practical experience of years of food testing and detailed statistical analysis of test results, Allermetrix continues to evolve better testing strategies. We refer to this strategy as “**Applied Science**”. Only with continuous review and reevaluation can laboratories help physicians identify causes of food allergy and ultimately help those who suffer.

It is clear that physicians must correlate all results back to the patient clinical symptoms and history in order to obtain the correct diagnosis.

Individualized elimination/rotation diets are available at Allermatrix (based on your food selections). Additionally, individualized patient information for how to avoid any hidden food testing positive (e.g. egg, wheat etc. which are contained unknowingly in many food preparations) is provided.

Graphical Data of In-House Food Testing Used to Create the Allermatrix 900 Panel (15,000+ tests)



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