

## In Focus: IgG4 Food Antibodies

### Sensational Food Allergy

Food sensitivity or intolerance is a contentious topic for healthcare professionals and consumers alike. It is regularly the subject of intense media speculation and scrutiny. This may be due in part to the clouded definition and misinformation of what actually constitutes a food sensitivity or intolerance. By contrast, the typical immediate allergy response to shrimps or peanuts, for instance, is a well characterized phenomenon; classified as a Type I allergy. In certain individuals Type I allergies can be potentially fatal.

For the purpose of this article, we will classify adverse reactions to food according the following definitions<sup>1</sup>.

- 1) Food allergy: A response mediated by food-triggered basophil or mast cell histamine release. This can be caused by either IgG or IgE food-specific antibodies. These reactions are immediate in nature and can be severe. This is the Type I allergic reaction.
- 2) Food sensitivity: a purely immune system-mediated response involving various classes of food-specific immunoglobulin molecules that can form food immune complexes. These complexes can stimulate the complement cascade and localized inflammation. These reactions tend to be delayed-hours up to 7 days after food consumption in some cases. This is a Type III allergic reaction.
- 3) Food intolerance: a non-immunological mechanism of adverse food response. Examples would include lactose intolerance and MSG sensitivity.

### In The News Again

#### Which IgG Test – 1, 4 or All??

The misinformation and lack of mechanistic explanations about IgG-mediated food sensitivity is reflected in the number of different IgG tests available to healthcare practitioners. Tests measuring total IgG subclasses (i.e., 1 through 4), IgG1, IgG4 or both IgG1&4 are available. During their tenure at university or medical school, most doctors and naturopaths are not educated on the properties of the different IgG subclass antibodies; which ones activate the complement cascade; or their behaviour after continued antigen exposure. Such information is vital when determining the most appropriate IgG subclass antibody to measure for assessment of IgG-mediated food sensitivity.

#### Subclasses Explained

There are four subclasses of IgG, 1 through 4, of which IgG1 and IgG4 appear to be the most dominating subclasses to food antigens<sup>2</sup>. IgG1 antibodies are the initial IgG class responders to a new food antigen. IgG2 and IgG3 are generally not produced to food antigens. Instead they react to cell surface oligosaccharides of viruses and protozoa, respectively. Once IgG1 binds to the antigen, the antibody-antigen complex is quickly destroyed by the Kupffer cells in the liver and other macrophages. The IgG1-antigen complex can also stimulate the complement cascade and attendant inflammation. This cascade of events is associated with the general malaise experienced from the inflammatory response<sup>1</sup>.

### **IgG4 Immune Complex Formation**

Upon continued exposure to the antigen, it is proposed that IgG1 antibody production will “class switch” to IgG4<sup>3</sup>. Interestingly, IgG4 antigen complexes do not activate the complement cascade. IgG4 acts as a blocking agent against the actions of IgE and can form small complexes as antigen exposure increases<sup>4</sup>. These IgG4 food immune complexes have a relatively long half-life and are subject to alterations that would affect the structure enough to present as a “new” antigen<sup>5</sup>. It is thought that IgG1 is then produced to attack this complex. Thus begins a whole new cycle: IgG1 › IgG4 › c omplex › modification › IgG1 › IgG4. Consequently, the complexes can get larger and larger. These larger complexes can activate the complement cascade, initiating inflammatory responses in the body. It is this inflammatory response to a food that is thought to be the root cause of symptoms in this type of adverse food reaction. The symptoms resulting from food sensitivities, therefore, can come from the activation of complement via IgG1/IgG4 food immune complex. Deposition of these complexes can also occur in tissue or organs, leading to damage. This sequence of events is thought to be the most common way individuals develop adverse reactions to foods they eat on a regular basis<sup>1</sup>.

In support of the above theory, researchers from Norway recently published findings suggesting a particular role for IgG4 in patients with delayed, non-IgE-mediated cow’s milk allergy (CMA)<sup>6</sup>. The authors measured beta-lactoglobulin (b-LG) specific IgE, IgG, IgA, IgG1 and IgG4 levels in both clinically reactive and tolerized IgE-mediated and non-IgE-mediated CMA patients. Compared with tolerized patients, levels of b-LG-specific IgG4 levels in clinically reactive CMA patients with non-IgE-associated delayed gastrointestinal symptoms were significantly higher. The inference from these findings was that IgG4, rather than total IgG or IgG1 is involved in the immunopathological mechanism in patients with delayed CMA.

### **Issues with IgG1 and Total IgG**

IgG1 antibodies tend to be more “sticky” and can bind more non-selectively to antigens, leading to a greater chance of cross-reactivity and false-positives; for example, watermelon and ragweed are cross-reactive. Measuring both IgG1 and IgG4 together can cause many unnecessary food eliminations. The IgG4 antibody is, therefore, a more clinically relevant marker of chronic food-immune reactions and possible intestinal permeability. IgG4 measurements are less likely to produce false-positives on in vitro tests. In a similar fashion, measurement of total IgG tends to produce a high rate of false-positive reactions.<sup>1</sup>

### **The Metamatrix IgG4 Food Antibody Assay**

Aware of the general criticisms of IgG food antibody testing in the industry, Metamatrix have developed a patent pending novel technology in an attempt to prove critics wrong. Metamatrix now provide a very accurate, sensitive and specific assay for the detection of IgG4 antibodies in blood. Similar to other laboratories, Metamatrix use the sandwich ELISA method, however the assay differs in the following respects:

- Metamatrix use three different methods to increase sensitivity by decreasing background and non-specific binding (i.e., no false positives)
- Metamatrix use a highly purified mouse monoclonal antibody to detect the IgG4 antibodies bound to the antigens attached to the plate. Studies on this monoclonal antibody by the Clinical and Laboratory Standards Institute (CLSI, formally NCCLS) guidelines revealed little or no cross-reactivity to purified human IgA, IgE and other subclasses of IgG. It was well below the recommended target of 0.001%
- Metamatrix use a novel signal amplification system that reduces the amount of serum required to perform the assay (reduced from 2 ml to 30 ul)

### **Testing In Practice**

Given the important differences in the properties of IgG subclasses outlined above, practitioners using the Metamatrix IgG4 food antibody test may report fewer reactions than when using a test which reports total IgG subclasses. A reduced incidence of false-positives allows for more targeted, relevant results that can improve patient compliance and outcome. Patients are not encouraged to eliminate from their diets so many foods that are not truly causing delayed sensitivity reactions.

## References

1. Lord, RS & Bralley, JA 2008, Laboratory Evaluations for Integrative and Functional Medicine, Metamatrix Institute, Duluth.
2. Host A, et al. Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow milk allergy. Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow milk allergy. *Allergy*. 1992;47(3):218-229.
3. Snapper, C. M.&Finkelman, F. D. (1999) in Fundamental Immunology, ed. Paul, W. E. (Lippincott, Philadelphia), pp. 831-861.
4. Meiler F, et al. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. *Allergy*. 2008;63(11):1455-63.
5. Larsson PH. Purification of antibodies. *Methods in Molecular Medicine*. 2008; 138: 197-207.
6. Sletten GB, et al. Changes in humoral responses to b-lactoglobulin in tolerant patients suggest a particular role for IgG4 in delayed, non-IgE-mediated cow's milk allergy. *Pediatric Allergy and Immunology*. 2006;17(6):435-443.