

# A Critical Review of IgG Immunoglobulins and Food Allergy – Implications in Systemic Health

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Food allergy is defined as an adverse clinical reaction due to an immune-mediated hypersensitivity response resulting from the ingestion of a food. A wide variety of foods have been shown to produce allergic reactions including cow's milk; chicken eggs; legumes; fish and shellfish; and cereals.<sup>1</sup> Depending on the speed of onset of symptoms, less than 45 minutes to 2 hours to days, immediate and delayed food allergies have been described throughout the literature encompassing a variety of gastrointestinal, respiratory, and cutaneous pathologies.<sup>2</sup> The inflammatory response is the common theme to all allergic pictures and is characterized by the release of chemical mediators, vasodilation, increased vascular permeability, edema, and tissue damage.

The role of IgE in Type I immediate hypersensitivity allergic reactions is well understood in the scientific literature. In classic Type I IgE-mediated hypersensitivity, food-specific IgE antibodies bind to FcεRI and FcεRII receptors on the cell membranes of mast cells, basophils, macrophages, monocytes, lymphocytes, eosinophils and platelets. Inflammatory mediators including histamine, serotonin, and tumor necrosis factor alpha, are released and induce symptoms upon exposure of these bound antibodies with food antigens that have penetrated the protective intestinal mucosal barrier. It is generally understood that symptoms of an IgE-mediated allergy manifest within 2 hours of consumption of the culpable food. Classical atopic symptoms include; urticaria, eczema, respiratory and nasal symptoms, and gastrointestinal distress. In the gut specifically, mast cell degranulation and mediator release promotes muscle contraction, stimulates pain fibers, increases mucus production, recruits inflammatory cells, and increases permeability to macromolecules, the latter of which may perpetuate a vicious cycle of food antigen exposure and symptoms.<sup>3</sup> Specific IgE has a half-life of only 1-2 days in circulation however, exhibits residual activity on mast cells of about 2 weeks with late phase reactions and inflammatory changes.<sup>4</sup>

It is strongly argued in the scientific literature that allergic reactions may occur independent of antigen-specific IgE. High affinity receptors for IgG (FcγRI), on human mast cells and basophils, are activated in immediate hypersensitivity reactions, following receptor aggregation through IgG binding. IgG-mediated immediate hypersensitivity results in degranulation, with the release of histamine and arachidonic acid metabolites.<sup>5</sup> Okayama et al, have demonstrated that the mediator profile through

activation of FcγRI receptors on human mast cells, is qualitatively indistinguishable from responses stimulated through FcεRI, the high affinity receptor for IgE.<sup>6</sup> In addition, FcγRI receptor expression on mast cells is up regulated by IFN-gamma, allowing for recruitment of mast cells through IgG-dependent mechanisms into the IFN-gamma-rich inflammatory locus.<sup>7,8,9</sup> IgG-mediated immediate hypersensitivity, also known as IgG-mediated anaphylaxis, is not a new concept in allergy research. In conventional circles, anaphylaxis denotes an immediate hypersensitivity reaction to an allergen, exclusively mediated by IgE antibodies. Hence, the foundation of the skin-scratch testing method, which detects IgE –induced histamine release through a wheal and flare response from antigen provocation, and IgE RAST quantification. However, as early as the 1970's, Parish demonstrated the presence of anaphylactic IgG antibodies in human sera.<sup>10</sup> Halpern et al, later suggested that this IgG anaphylactic antibody is indeed a subtype of IgG4. Further studies from Bryant et al, and Pepys have shown that IgG anaphylactic antibody activity could not be removed through precipitation with anti-IgE but, only by precipitation with anti-IgG, clearly indicating a novel mechanism for mast cell recruitment into inflammation.<sup>11,12</sup> However, the potential for IgG4 to inhibit, or block IgE-mediated anaphylaxis is a clearly established theme in this line of research, and some authors argue a correlation between increased IgE levels and IgG4 in atopic patients, where IgG4 is thought to hamper antigen binding to cell-bound IgE which would otherwise promote a much stronger allergic reaction. Moreover, the basic principle behind allergen immunotherapy (IT), is oral or intradermal administration of the allergen to induce the development of a systemic immune response, including the production of systemic blocking antibodies. In an update on immunotherapy, "Immunotherapy update: mechanisms of action", Greenberger concludes that the reduction of allergic symptoms, specifically of allergic rhinitis and asthma, reflect changes in the cytokine and immunoglobulin profile from intradermal allergen provocation. Most notably, intradermal grass injection resulted in a profound increase in anti-allergen IgG (2-10 fold), IgG4 (10-100 fold), a decline in anti-allergen IgE antibodies, reduced numbers of nasal or bronchial mast cells and eosinophils, down-regulation of T-helper 2-lymphocytes and IL-4, and a lack of increase in interferon gamma.<sup>13</sup> This study clearly establishes IgG4 as a blocking antibody. Furthermore, in a study involving 42 children with malabsorption disorders, those demonstrating high levels of IgG antibodies to ovalbumin (Anti-OA) showed

significantly lower serum concentrations of OA at both 2 and 8 hours after oral OA administration, compared to children with lower anti-OA levels. The researchers conclude that the high levels of IgG antibodies to ovalbumin demonstrate blocking capacity in the circulation. Such antibodies in the intestinal secretions and in the gut wall would limit the quantity of antigen that can penetrate the mucosa and enter circulation.<sup>14</sup> However, other studies show elevated IgG4 in symptomatic atopic patients without a concomitant rise in IgE levels.<sup>15,16,17</sup>

Supporting evidence from Yoshida et al, demonstrate that IgG4 is not only elevated in milk allergic children, for example, but diagnostic of milk allergy in atopic children independent of IgE.<sup>18</sup> In another study, milk-specific IgG4 in particular, IgG4 to casein, has been shown to be diagnostic of milk allergy causing eczema in adults.<sup>19</sup> In an elegant study by Eysink et al, atopy could be correctly classified in 75.4% of young children studied with or without eczema, through identification of high levels of IgG to certain foods. In particular, high levels of IgG antibodies to egg, milk, orange, and a mixture of wheat and rice, were identified in atopic children compared to nonatopic children. Further, this elevation served as a positive predictor of increased IgE antibodies to inhalant allergens, namely cat, dog, and house dust mite. The investigators of this study conclude that the association drawn from these results may clearly identify children with an increased risk of developing future allergic disease.<sup>20</sup>

Other reports demonstrating the importance of IgG antibodies in food allergies include IgG-mediated allergy to casein and other milk proteins, which has been implicated in the development and progression of infantile autism.<sup>21</sup> Furthermore, one study involving rheumatoid arthritis, showed a decrease in gluten-specific IgG serum levels which correlated with an improvement in the symptoms of this disease in 40% of subjects placed on a gluten-free diet, compared to a 4% improvement in a control group, over a one-year period.<sup>22</sup>

The evidence from the above research suggests that IgG4 antibodies may act as sensitizing as well as blocking antibodies. This dual role of IgG4, anaphylactic or blocking antibody, lends way to defining IgG4 subtypes 4a and 4b, as described by Halpern and Scott in their review, "Non-IgE mediated mechanisms in food allergy", whereby exposure to an allergen may lead to the production of the anaphylactic or nonanaphylactic/blocking subtype of which may depend on genetic predisposition and environmental factors.<sup>23</sup> It is interesting to note that there is some structural homology between IgG4a, IgG1 and IgG3 immunoglobulins, and between IgG4b and IgG2.<sup>24</sup> IgG subclass antibodies and their role in the pathogenesis of food allergic disease deserves considerable attention. Chronicity of antigen exposure, a hyperactive mucosal immune system and/or an increased permeability to

macromolecules, are factors to consider influential in IgG4 subclass expression and progression of disease. A distinguishing feature of IgG4 is its inability to activate the classical complement pathway. This supports the role of IgG4 as a blocking antibody. In a study of egg hypersensitivity, Nakagawa draws our attention to IgG1 involvement in clinical egg hypersensitivity, suggesting that increased IgG4 reduces the effect of complement-fixing antibodies like IgG1; a good prognostic sign as he suggests.<sup>25</sup> This is further supported by Van Der Zee, who shows that IgG4 antibodies inhibit complement activation of IgG1 antibodies, probably through competitive binding for related antigenic determinants.<sup>26</sup>

Like all immune mediated reactions, food allergies depend on the intimate association between mature T-helper cells and B-lymphocytes with the production and release of inflammatory mediators and activation of food specific antibodies. After B-cells are stimulated by antigen, they are terminally differentiated into plasma cells of which secrete antigen-specific immunoglobulins.

A review of T-helper 2 (TH2) and T-helper 1 (TH1)-induced antibody production reminds us that interleukin-2 (IL2), interferon gamma (IFN gamma), and tumor necrosis factor beta (TNF beta), via TH1 T-helper cell activity, favors B-lymphocyte class switching to the production of IgG2a. Conversely, IL-4 and IL-5 secreted by TH2 T-helper cells induce class switching to IgE and IgG1. The exact role of the different subclasses of IgG remains to be understood. Studies suggest sequential switching as a prerequisite for B-cell differentiation, most prominent in cells that switch to IgE. For instance, B-cells cultured with interleukin-4 show IgG1/IgE double-positive staining, which appear after 3 days of culture, after which predominantly secrete IgE on reculture. This serves as an interesting note suggesting an essential switch from IgG1 with possible prerequisite involvement in IgE production.<sup>27</sup> The amount of antigen absorbed, the quantity of antibody required, the chronicity of antigen exposure, and the specific role of IgG subclasses in the pathogenesis of food allergic illness, clearly influence progression of disease, and define the magnitude of the immune response to dietary antigen to warrant further investigation.

A key feature of food allergies that deserves close attention is its implications in the development and maintenance of immune cell memory from chronic and repeated exposure to food allergens, with resultant clinical consequences. Food-induced immune reaction favors maturation and proliferation of naïve T-cells into CD4 and CD8 effector T-cell lines. Under chronic antigen exposure CD4 populations generate distinct populations, as mentioned above, TH1 and TH2 of which TH2 promotes the activation of cytokines favoring immunoglobulin production. From chronic antigen exposure there is a pronounced alteration in the ratio between TH1 and TH2, suggesting immune imbalance, polyclonal B-cell

activation, and an exaggerated immunoglobulin response. This mechanism of action has been argued for autoimmune disease due to chronic antigen exposure, and has interesting implications in food allergies, which also represent a state of chronic antigen exposure. As such, the role of IgG in delayed-onset food allergies deserves close attention for its implications in systemic disease.

There is no argument regarding the presence of serum IgG reactive with different dietary proteins. Specific serum IgG antibodies to different food proteins have been reported in significant numbers of adults and children in cases of celiac disease, dermatitis herpetiformis, and atopic eczema.<sup>28,29</sup> Moreover, higher total and specific serum IgG4 levels to common foods are raised in cases of atopic eczema compared to the healthy population.<sup>30,31</sup> The presence of elevated levels of IgG antibodies to food antigens have been observed substantially in diseases with increased intestinal permeability, in particular, IgA deficiency<sup>32</sup> and inflammatory bowel disease.<sup>33</sup>

The role of secretory IgA is clear; induction of mucosal immunity and establishment of oral tolerance through oral immunization with food antigens occurs in most normal individuals, and plays a major role in antigen handling and elimination. The elevation of IgG in IgA-deficient states suggests increased intestinal permeability to macromolecules. In other words, a lack of sIgA may permit the permeation of undigested food antigens into the blood stream, thereby allowing for immune complex formation and circulation for an uncertain period of time. Cunningham et al, conclude from their study involving IgA-deficient subjects, that milk and other protein precipitins are a common feature in IgA deficiency, and that a high proportion of these individuals may have circulating immune complexes of these food antigens.<sup>34</sup> In addition, a review by Saavedra-Delgado and Metcalfe explain from other studies, that the presence of circulating IgG-food antibodies is consistent with increased absorption of food antigens and stimulation of antibody production, as a result of mucosal damage. These authors note that circulating antigen-antibody complexes are more frequently detected in symptomatic cases of ulcerative colitis as evidenced by IgG - immune complex deposition and complement in colonic epithelium along the basement membrane.

It is not difficult to conceive a rationale for the observed elevation in IgG antibody levels to food antigens in IgA-deficient states, when we consider the mechanisms of oral tolerance. Oral tolerance lies at the heart of immunological theories and is the cornerstone of setting up a reaction or non-reaction against self and non-self (dietary challenge). Factors influential in the induction of an immune response with IgE and IgG expression, versus the induction of active suppression, or immune tolerance, an IgA response, are cutting edge research and are under considerable investigation. Oral tolerance to dietary antigens via TH3-

cell activity, or suppressive intra-epithelial lymphocytes (IEL), is the B-cell switching from IgE/IgG antibody production to IgA, under the influence of a novel cytokine profile unlike that governing TH1/TH2 mechanisms. In particular, transforming growth factor beta (TGF-B), the predominant oral tolerance cytokine, is released by activated IEL's and suppresses any potential for TH1 or TH2 response to dietary antigen, thereby favoring IgA expression and active suppression. Bias towards immune response TH1/TH2, or immune tolerance TH3, is dependent on the cytokine profile elaborated under the influence of the gastrointestinal mucosal immune milieu, defined by the individual's defense factors, innate and acquired. It is especially important to note that the immune response favored in the adult will differ from that of the neonate, where there is a predilection towards oral tolerance in the latter. In any event, local gastrointestinal immunity, through the expression of a cytokine profile indicative of either an immune response or active suppression, each being unique, will have systemic consequences, as these cytokines migrate to other mucosal sites and peripheral tissues, with implications in the onset and progression of symptoms.<sup>35</sup>

It is proposed that the breakdown of oral tolerance, and hence sensitization to dietary antigens may occur early in life during a viral or bacterial infection, under conditions where the secretory immune system has not fully matured. By that means, a hyperresponsive state to dietary agents is set up, with inflammation and the development of inflammatory bowel disease; ulcerative colitis or Crohn's disease.<sup>36</sup> By definition, inflammatory bowel disease is the loss of tolerance to normal flora with consequential hypersensitive food reactions and systemic sequelae.<sup>37</sup> These arguments are supported by many authors including Tahmeed and Fuchs, who comment in their review that intestinal infections and reduced secretory IgA may alter intestinal permeability resulting in an increased uptake of food antigens thereby initiating an abnormal mucosal immune response and chronic enteropathy.<sup>38</sup> The breakdown of oral tolerance and resultant disease is far reaching and characterizes a number of conditions including childhood onset Type I hypersensitivity, celiac disease, and a number of autoimmune conditions.<sup>39</sup>

Mucosal exclusion of dietary antigens other than via secretory IgA has also been demonstrated to occur through experimentally induced high titers of IgG antibodies. However, some in vitro experiments using everted gut sacs show the contrary, with enhanced intestinal absorption of antigen under the influence of high IgG titers.<sup>40,41</sup> Tolo et al, through in vitro studies of rabbit oral mucosal membranes, found that serum-derived IgG antibody retards the penetration of corresponding antigen, however impairs the mucosal barrier none the less, by allowing for concurrent mucosal penetration of unrelated macromolecules.<sup>42</sup> It is not unreasonable to rationalize a similar set of circumstances for intestinal epithelia given

sufficient experimental data. Immunohistochemical studies demonstrate an abundance of serum-derived IgG present in the lamina propria of mucous membranes, traces of which diffuse into the intestinal epithelial interstices. Immune complex formation in these sites may on the one hand, perturb mucosal antigen uptake by signaling the emigration of neutrophils with phagocytosis and protein degradation. On the other hand, the local emigration of other immune cells and the release of inflammatory mediators may concomitantly enhance mucosal permeability and penetration of other food macromolecules. As such, IgG may in effect compromise mucosal integrity through undue penetration of new food antigens thereby setting up a vicious cycle. To review, by activating complement, IgG antibodies may promote increased mucosal permeability, tissue damage, and persistent immunopathology. In addition, IgE antibodies may enter the gut mucosa via mast cells causing their degranulation with histamine release and mucosal lesion formation as well. Such immunological mechanisms may therefore perpetuate an inflammatory state of the bowels with undue systemic exposure to dietary antigens.<sup>43</sup>

It is important to note that intestinal hyperpermeability is not necessarily a prerequisite for penetration of food antigens into the lamina propria and systemic circulation. Kleinman and Walker mention that small, nutritionally insignificant amounts of antigenically intact macromolecules may be transferred across the gut lining through simple mechanisms.<sup>44</sup> With this in mind, when we consider the absorption of pounds of foods on a daily basis, continuous exposure between food antigen, most often of the foods eaten regularly, and stimulation of intestinal lymphoid follicles may very well provide the impetus for the development of a systemic immune response with circulating antibody complexes and atopic reactions.

In Type III, immune complex-mediated hypersensitivity, IgG antibodies combine with food antigen forming circulating immune complexes to which complement is fixed. These immunocomplexes may circulate throughout the periphery and deposit in various tissues promoting an Arthus-like inflammatory reaction with vasculitis and tissue damage. Intestinal biopsy studies have shown evidence of this type of immune-mediated reaction in the pathogenesis of cow's milk sensitive colitis.<sup>45</sup> In another study, Lee et al, have demonstrated deposition of human IgG and precipitins to cow's milk in lung tissue specimens of infants with pulmonary hemosiderosis. Immunofluorescence of snap-frozen biopsy tissue in these infants exceeded that in control lung biopsies. The researchers suggest a Type III mediated mechanism to explain the presence of the milk-related pulmonary infiltrates. Absorption of the milk antigens through the intestine with subsequent deposition of the circulating immune complexes could not be discounted as there was no evidence to suggest aspiration, and symptoms appeared

within the hour following consumption of cow's milk with notable immune complexes appearing in the serum.<sup>46</sup>

Since immunocomplexes, through Type III hypersensitivity are free in circulation, the implications of multiple end organ pathology are not without possible justifications. It may be possible to correlate such phenomena to the pathogenesis and etiology of certain autoimmune disease<sup>47</sup>, connective tissue disorders, and perhaps malignancy.

In addition to IgG involvement in Type I and III hypersensitivity reactions, there are concrete studies demonstrating the role of specific IgG antibodies to food allergens in Type II immune mechanisms as well. In Type II, antibody-dependent cytotoxic hypersensitivity, specific IgG recognize and bind to food antigens that have adhered to the surface of cells. Antigen-antibody bound complex activates the complement cascade and the release of cytotoxic substances from activated killer cells, with eventual cell death. Cow's milk-induced thrombocytopenia has been implicated by this type of reaction.<sup>48</sup>

Through Type IV, cell-mediated delayed hypersensitivity, IgG antibody activity plays an integral role in this type of immune response as well. Type IV mechanisms represent a major immunological pathway in conditions such as cow's milk-induced enteropathy and celiac disease.<sup>49</sup> Food induced gastroenteropathy, specifically celiac disease, has been clearly defined through Type IV mechanisms with the involvement of IgG specific antibodies to wheat gliadin. In this condition, abnormalities in intestinal permeability are associated with both inflammatory processes and loss of jejunal microvilli favoring the absorption of large molecules that pose an allergenic threat. Kemeny et al, have demonstrated increased IgG1 antibody levels to gliadin, ovalbumin, and casein in addition to elevated IgG4 to casein in untreated celiac patients compared to healthy control subjects.<sup>50</sup> In addition, in immediate-type egg allergic patients, these investigators found raised IgG1 antibody levels to ovalbumin, compared to healthy control subjects.<sup>51</sup> In the celiac subjects, in particular, both IgG1 and IgG4 antibody levels to gluten fell from a gluten-free diet.

As a clinical aside, anti-gliadin IgG antibodies are a sensitive screening test for jejunal biopsy in patients with suspected celiac disease. Also, the monitoring of the disappearance of gliadin antibodies during a gluten-free diet can be used to indicate successful elimination of gluten from the diet.

Symptoms of delayed food allergies are diverse and may affect any system in the body. It can be argued that delayed-onset food allergies are much more common than the more widely accepted IgE-mediated immediate hypersensitivity reactions. In addition, IgG-mediated food allergies may account for variety of chronic health

conditions that have otherwise been misdiagnosed and thus unresponsive to conventional medical care. Fatigue, irritability, aching joints, cognitive dysfunction, and chronic migraines are a few known complications due to food allergies, which suggest a strong IgG component due to their chronic nature. The diagnosis of food allergy is made simple though serum ELISA analysis for IgG-specific food antigens. The condition is treated by eliminating the allergenic food from the diet for an indefinite period of time.

Typically, IgG subclasses exhibit a half-life of about 21 days with a residual time on mast cells of about 2 to 3 months.<sup>52</sup> However, due to the long survival of IgG relative to other serum immunoglobulins, immune complex formation may persist in circulation for an indefinite period of time. As such, the time period required to abstain from the allergenic foods is arguable, and may extend for up to 9-12 months as in the case of cow's milk allergy.<sup>53</sup>

The production of antibodies to dietary antigens, especially of the IgG class takes place as an immune response to food antigens. This is true and well established through food allergy provocation studies and quantification of IgG via ELISA methodology. Effector function of the different subclasses varies. Chronicity of food antigen exposure, gastrointestinal mucosal integrity, and host immune competence, are a few of the physiological circumstances that influence subclass IgG expression, activation of complement, and cellular immune mechanisms. Together, these functional components of the mucosal immune system orchestrate progression or neutralization of the inflammatory process induced by food antigen exposure, the former of which may promote varying symptomology.

***An appreciation of the associated symptoms of food allergy poses a unique challenge to the clinician both because of the variability in severity and onset. As such, food allergy should not, by any means, be underestimated as a key etiological factor in disease and disease progression. Gastrointestinal food allergy is a fascinating line of research that merits close consideration in clinical practice for the assessment and care of our patients.***

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