Food-Specific IgG Antibodies:

In Health and Disease

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Introduction

It has been estimated that food intolerance, including IgG antibody-mediated reactions and related diseases, affect at least 100 million people worldwide and have increased by more than 50% in adults and children in recent years. According to recent data, IgG-mediated food sensitivity, characterised by a delayed immune response, have been shown to play a significant role in the pathogenesis of adverse food reactions. As such determination of serum food-specific IgG antibodies potentially opens new diagnostic pathways for patients who are hypersensitive to food components, where resulting symptoms can be alleviated by food elimination diets based on the results of such testing [1]. A greater understanding of this type of hypersensitivity reaction is warranted, not only from an educational perspective, but also to ensure that where it is appropriately applied, more individuals can be helped in achieving relief from a range of difficult to treat chronic symptoms.

The specific characteristics of IgG-mediated food sensitivity, results in a delayed nature for such reactions, which is a considerable diagnostic obstacle, making it almost impossible for the patient or clinician to identify the factor(s) causing the reaction [2]. While IgE antibodies are responsible for acute, immediately developing allergic reactions, IgG-mediated reactions take much longer to develop up to 72 hours after ingestion of triggering foods. These antibodies play a significant role in the shaping of the body's normal immune response. The binding of IgG with a bacterial or viral antigen result in antigen coating and formation of immune complexes. The formation of the immune complex triggers further immune responses: activation of complement and stimulation of the release of pro-inflammatory cytokines (IL-1, IL-6, TNF-α), proteolytic enzymes and free radical pathway enzymes. The developing inflammation is accompanied by mechanical damage to the surrounding tissues. The activity of IgG is identical as far as food antigens are concerned. As the food components in patients with increased intestinal permeability enter the bloodstream from the intestinal lumen on a continuous basis, the immune mechanisms undergo constant activation, overloading the immune system's ability to clear such complexes efficiently, which results in activation of low-grade inflammatory pathways.

Evidence suggests that the development of IgG-mediated food sensitivity is directly related to increased permeability of the intestinal barrier [3]. In a healthy normal intestinal tract where homeostasis is preserved, this layer is tight and highly selective, so that only the desired nutrients enter the bloodstream from the intestinal lumen, while access of potentially damaging substances and pathogens is impeded. This barrier also plays a role in immune sampling and processing of potential antigens and in the prevention of infection (e.g. the binding of pathogens by secretory IgA (sIgA)). The main structural elements of the intestinal barrier responsible for its correct functioning include commensal microorganisms, secretory IgA, enterocytes along with their healthy tight junctions, Peyer's patches, M cells, antigen presenting cells (APCs) and lymphocytes. Studies have shown [4, 5, 6] that damage to the tight junctions between the enterocytes is the precipitating cause for the development of food-specific IgG antibodies. Correctly functioning tight junctions between the intestinal cells ensure that the barrier shows the required selectivity.

Elevated levels of IgG antibodies to food antigens have been observed frequently in diseases associated with increased intestinal barrier dysfunction, in particular IgA deficiency, coeliac disease and inflammatory bowel disease (IBD) [7-9]. The controversy surrounding food-specific IgG testing relates to the significance of food-specific IgG antibodies in the pathogenesis and diagnosis of IgG-mediated food sensitivity and its relationship to chronic illnesses, including IBD, IBS and migraine. While more research is required, clinical studies to date, have largely been supportive of a role for food specific IgG antibodies in certain illnesses.

Background

An adverse food reaction is a general term describing clinically abnormal responses to an ingested food that may be related to both immune and non-immune mechanisms. Food allergy is an immunologic reaction that typically involves the immunoglobulin E (IgE) mechanism, of which anaphylaxis is the classic example. Such reactions are classified as a Type I hypersensitivity as defined by Gell and Coombs in 1963 [77] (fig 1). Measurement of food-specific IgE antibodies by *in vitro* assays or skin testing are the routine procedures used to diagnose food allergy. These diagnostic tests, however, indicate the presence of food-specific IgE antibodies, but they do not definitively establish the diagnosis of food allergy on their own. Confirmation of the clinical relevance of the reported history and the detected food-specific IgE antibodies is provided by a positive controlled food challenge [10].
Position statements and reviews of allergy that are referring to IgE-mediated reactions should not be confused with IgG reactions, as total IgG (subclasses 1-4) assessments do not have any diagnostic relevance to the investigation of this type of allergy (IgE). This point is frequently confused and misunderstood in publications and online reviews.

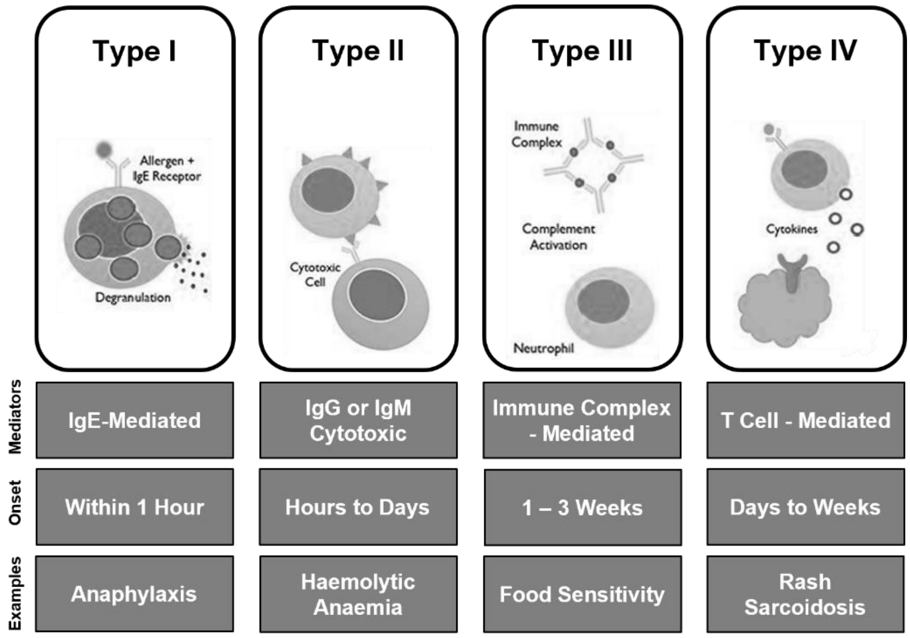


Fig 1. Classification of hypersensitivity reactions

Food intolerance, however, is a general term, describing an abnormal physiologic response to an ingested food or food additive. Such reactions are considered to not demonstrably involve the immune system, an example being lactose intolerance where there is a deficiency in the enzyme responsible for breaking down ingested lactose. However, these classifications fail to include reactions mediated by immunoglobulin G (IgG), which since such reactions do involve the immune system, would be classified as a form of allergy, and regarded as an example of a Type III hypersensitivity reaction (fig 2). This has led to a great deal of confusion amongst lay persons and professionals alike where IgG-mediated reactions are often referred to as food intolerance or where IgG-mediated reactions are referred inappropriately as 'allergy'. Food sensitivity is a term alternatively used to distinguish such reactions from classical allergy (IgE) and food intolerance (non-immune) to avoid confusion, throughout this document we will refer to these types of reactions as IgG-mediated food sensitivity.

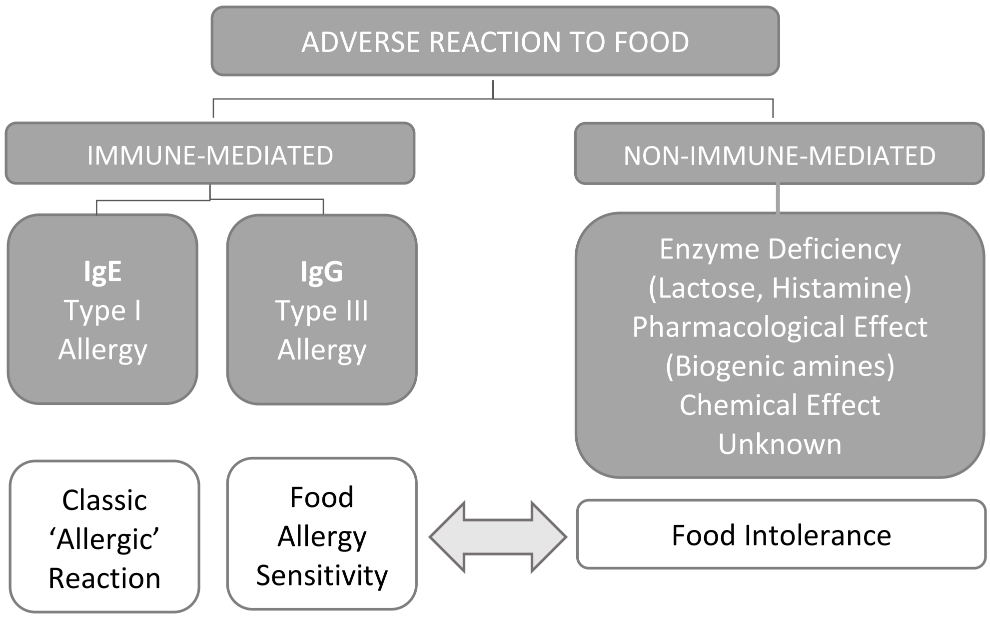


Fig 2. Simplified classification of food reactions

IgG-mediated food sensitivity is a distinct entity, resulting in very different clinical pictures. The reactions are usually defined as 'delayed' with a less severe symptom outcome compared to some IgE-mediated reactions. Symptoms can typically affect many different body systems and as they are regarded as an example of a Type III hypersensitivity reaction, are characterised by the production of immune complexes with food-specific IgG antibodies activating the complement pathway and thus initiating low grade inflammatory reactions [11]. Paganelli *et al* [12], demonstrated that ingested food molecules are at the core of such circulating immune complexes and pointed out that whilst *'The formation of an antigen-antibody complex in the circulation is a normal physiological method of antigen elimination, there is a great deal of evidence suggesting that increased levels of circulating immune complexes are associated with a variety of diseases in which the complexes, once deposited in the tissues, cause damage by activating complement and other effector mechanisms.'*

Clinical Manifestations

The clinical manifestations of IgG-mediated food sensitivity depend on the target tissue or organ to which the immune complexes composed of food-specific IgG antibodies and the corresponding food antigens are transported to via the bloodstream. High levels of the complexes accompany such dissimilar disease entities as migraine, IBS and inflammatory bowel disease. The signs and symptoms develop within 8–72 h or even longer after ingestion of the offending food. Delayed symptoms often mean that patients do not associate a given symptom with the food they ate, the mild severity of the clinical manifestations or their complete lack is generally associated with a considerable delay of the reaction in time or with low titres of IgG in the blood due to low permeability of the intestinal barrier.

This type of IgG-mediated sensitivity may affect various organs and systems, such as the gastrointestinal tract (nausea, vomiting, diarrhoea, abdominal pain), respiratory tract (rhinitis, sneezing, itchy throat, laryngeal oedema, hoarseness, cough, dyspnoea, asthma) and neurological system (headache or migraine).

Conditions in which elevated food-specific IgG antibodies have been associated

- IBS [13]
- Inflammation, hypertension & arthritis [11, 14]
- Migraine [15, 16]
- Asthma / respiratory diseases [17]
- Crohn's disease [18]
- Schizophrenia, bi-polar, autism and major depressive disorder [19, 20, 21]

Immunological Mechanisms

The breakdown in oral tolerance and hence sensitisation to dietary antigens has been and continues to be the subject of intense scientific research worldwide and considerable progress has been made in elucidating the role played by dietary proteins and the antibodies directed against them. Numerous studies have demonstrated:

1. That in recent years, mast cells play a role in both the physiological processes of the intestine and the pathophysiological factors of IBS: visceral hypersensitivity, dysmotility, mucosal barrier disruption, altered intestinal fluid secretion and mucosal immune dysregulation. The presence of degranulated mast cell rich low grade intestinal inflammation in patients with IBS has focused the attention to the IBS and mast cell association. Since the intestinal mucosa is mainly exposed to food and bacterial antigens, it is natural that food reactions due to mast cell activation may be part of this inflammation. Indeed, the authors of one paper suggest that this mechanism is the 'missing piece of irritable bowel syndrome puzzle' [80]. Antigen-specific IgG in the form of complexes can activate human mast cells and basophils, via high affinity receptors (FcγRIII), resulting in degranulation and the release of histamine and arachidonic acid metabolites. The mediator profile through activation of IgG receptors on human mast cells has been shown to be qualitatively indistinguishable from responses stimulated through the high affinity receptor for IgE [22, 23].
2. A role for food IgG antibodies in Type III, immune complex-mediated hypersensitivity. IgG antibodies combine with food antigen to form circulating immune complexes to which complement is fixed. Such complexes deposit in various tissues promoting Arthus-like reaction resulting in vasculitis and tissue damage [25]. Activation of complement primarily results in cleavage of soluble complement proteins forming C5a and C3a, which activate recruitment of PMNs and local mast cell degranulation (requiring the binding of the immune complex onto FcγRIII [22,23], resulting in an inflammatory response. Based on histologic and immunofluorescent findings in intestinal biopsies, there is evidence that the Arthus-type reaction is involved in the pathogenesis of cow's milk sensitive colitis [26
3. Increased intestinal permeability after oral challenge and a role for mast cells in the regulation of intestinal barrier dysfunction in patients with food allergy [28-32]. Whilst such studies focus on IgE-mediated allergy, the activation of mast cells through the activation of IgG receptors by food-specific IgG antibody immune complexes, as described above cannot be discounted, indeed the recent elucidation of novel mast cell activation pathways, have clearly implicated IgG as a potential trigger for such activation [33].
4. IgG receptor polymorphisms play an important role in the pathogenesis of inflammatory disease [34-37]. Aberrant regulation or function of these receptors in the presence of elevated food-specific IgG

antibodies would lead to magnified effector responses that initiate inflammatory disease and increased susceptibility to autoimmunity. Determination of food-specific IgG antibodies by ELISA provides a useful tool for patient-specific diet manipulation as means to control such IgG receptor-mediated effector functions in patients with receptor polymorphisms that are associated with disease susceptibility.

The biological mechanisms involved in IgG-mediated sensitivity and the role it plays in the onset of chronic diseases are clearly complex and remain to be fully elucidated. However, there is accumulating evidence from both clinical studies and scientific research that indicates that the effective assessment of adverse reactions to foods should include testing for food-specific IgG antibodies. Not only as a means to characterise the immunological response to dietary challenge in a patient but also to provide a rationale for the design of diet therapy.

Contact between food antigens and the immune system

In normal conditions, consumed proteins, including potential food antigens, are completely degraded in the digestive tract to oligopeptide fragments. Due to intestinal proteolytic enzyme activity, the latter are broken to di- and tripeptides and amino acids, and then absorbed by enterocytes. Enterocytes are cells which line the intestine and form an internal barrier. Further degradation takes place in enterocytes, to amino acids and dipeptides which enter portal circulation and then are carried to the liver. However about 15% of consumed protein is incompletely digested, including a proportion of food antigens. This is important as a certain amount of food antigens, which were not destroyed by digestion with enzymes, bile salts, and low gastric pH, penetrate the epithelium of the digestive tract and reach the body's internal environment [38].

There are three pathways for food antigens to penetrate the digestive tract epithelium [38, 39].

1. Capture of antigens by Peyer's patch M cells.
2. Capture of antigens from the digestive tract by the dendritic cell processes localized between enterocytes.
3. Capture of antigens by enterocytes.

When enterocytes are damaged and the connections between them weakened, for instance due to inflammatory processes, food antigens may fall between the cells. When this occurs, a far greater quantity of partially digested food antigens can penetrate the digestive tract. Here they encounter the cells of the immune system known as the gut-associated lymphoid tissue (GALT).
The role of GALT is to maintain the immune homeostasis between defending the organism from pathogens which have penetrated the digestive tract and inducing and maintaining the immune tolerance for innocuous antigens. Therefore, food antigens will be treated by GALT either as innocuous antigens and induce tolerance, or as pathogens. When treated as pathogens an immune response is triggered. This may be either a normal defence reaction or an excessive defensive reaction, the latter of which gives rise to IgG-mediated food sensitivity. [39].

The recent discovery of a new CD4+ T cell subset, Th17, has transformed our understanding of the pathogenetic basis of an increasing number of chronic immune-mediated diseases. Particularly in tissues that interface with the microbial environment—such as the intestinal and respiratory tracts and the skin—where most of the Th17 cells in the body reside, dysregulated immunity to self (or the extended self, the diverse microbiota that normally colonise these tissues) can result in chronic inflammatory disease.

Since the discovery of the Th17 linage IgG must be looked at from a different point of view than before.
IgG is not only the immunoglobulin that protects us against foreign infectious agents but is now also recognised as a mediator of inflammation and is responsible for auto-immune diseases. The balance of Th17 and Treg is largely responsible for the pro-inflammatory conditions in our body, both are expressed in peripheral tissue and in the gut in particular. Alterations of the microbiome and leaky gut induce the activation of the Th17 mediated immune response and the production of pro-inflammatory IgG antibodies against food and other potential harmful antigens present in the gut. Studies with mouse models demonstrate 2 pathways of systemic anaphylaxis: a classic pathway mediated by IgE, FcεRI, mast cells, histamine, and platelet-activating factor (PAF) and an alternative pathway mediated by IgG, FcγRIII, macrophages, and PAF. The alternative pathway by forming complexes with IgG that cross-link macrophage FcγRIII.

The role of IgG in triggering defence reactions to food antigens

The IgG antibodies are the main line of acquired defence and a body's specific humoral response to pathogens. In normal conditions, the digestive tract epithelium is impermeable to antigens. Whereas when it is damaged by inflammatory processes, antigens can permeate under the epithelium and contact immune system cells, which leads to immunisation and production of specific defensive IgG antibodies. The subsequent contact of these antibodies with the antigen causes defence reactions involving the creation of antigen-antibody immune complexes, activation of complement protein cascade and effector cells, such as neutrophils, lymphocytes, macrophages, as well as eosinophils and platelets. As a result, the immune complexes are phagocytosed and then destroyed in the reticuloendothelial system. Simultaneously, the inflammatory process caused by the immune reaction between IgG and food antigens might facilitate further damage and increased permeability of the digestive tract mucosa to food antigens. Therefore, the presence of specific IgG antibodies directed against food antigens reflects a defence reaction to antigens penetrating due to the damage of the epithelial barrier. The IgG response to food antigens reflects the damage to the mucosa and develops secondary to it and is also associated with the removal from the body of food antigens, which have penetrated the barrier of the mucosa, while the selectivity of response to certain food antigens may come from the type and quantity of a penetrating antigen and its resistance to digestion. This concept is well supported by the results obtained by Zuo *et al*, who investigated the concentrations of IgG against 14 food antigens in patients with IBS and functional dyspepsia, compared to a group of healthy patients. In all patients from both studied groups as well as in controls, the presence of IgG antibodies directed against food antigens was confirmed. Nevertheless, statistically significantly higher levels were observed in patients with IBS and dyspepsia than in controls. Simultaneously, the authors stress the selectivity of response to only some food antigens, which may be associated with dietary habits or other factors. The same study did not reveal any correlation between the severity of the symptoms of functional dyspepsia and IBS and IgG levels, while the total IgE concentrations in controls and studied groups did not differ statistically and were within normal ranges [1].

Increased intestinal permeability and food-specific IgG antibodies

Evidence suggests that the development of IgG-mediated food sensitivity is directly related to increased permeability of the intestinal barrier. In a healthy normal intestinal tract where homeostasis is preserved, this layer is tight and highly selective, so that only the desired nutrients enter the bloodstream from the intestinal lumen, while access of potentially damaging substances and pathogens is impeded. Loosening of the tight junctions makes it possible for larger particles – not only nutrients but also toxins, food antigens and microorganisms – to penetrate the barrier. The increased permeability of the intestinal barrier is often referred to as 'leaky gut syndrome' [6].

There is increasing evidence that damage to the tight junctions and the subsequent influx of toxins and antigens leads to abnormal immune reactions resulting in the development of autoimmune diseases. Several studies have also shown that this damage to the tight junctions is the precipitating cause for the development of food-specific IgG antibodies. Furthermore, there is evidence that this permeability is selective to food antigens. This was examined by Mansueto et al, in 2018 in a review of all publications relating to increased permeability, IgG antibodies and IBS. The authors concluded that the production of such antibodies is a specific reaction to food, rather than a non-specific reaction as is widely believed [40].

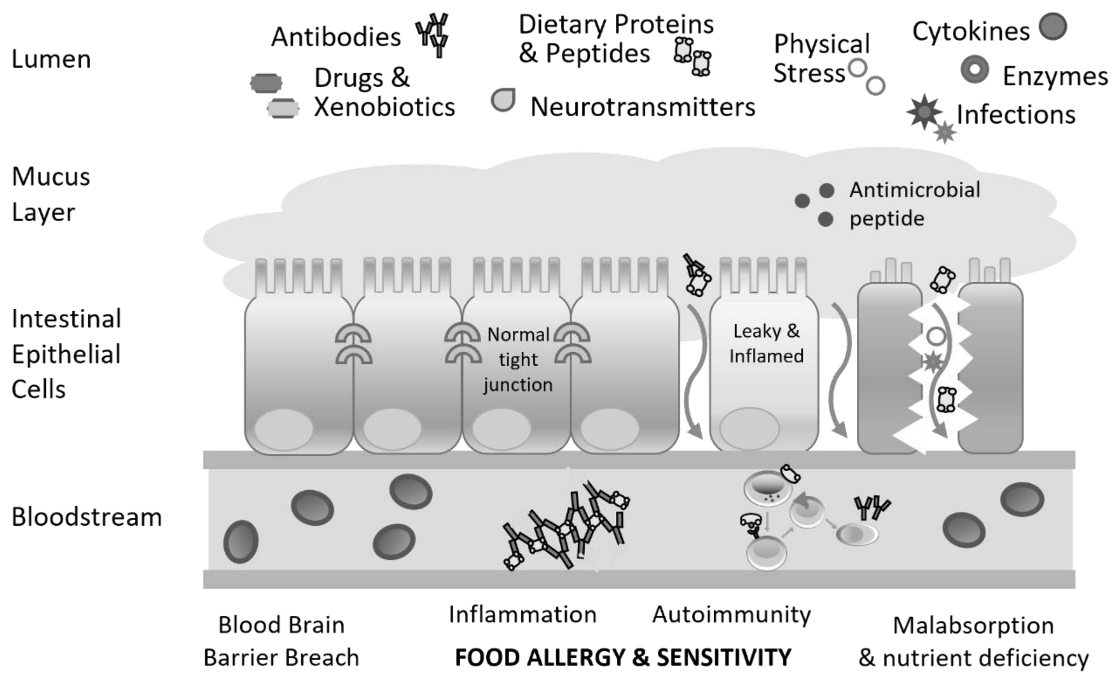


Fig 3. Consequences of abnormal intestinal permeability

Food-specific IgG antibodies and inflammation

Low-grade inflammation may play a causal role in the development of obesity, insulin resistance, diabetes mellitus and atherosclerosis [41]. In obese subjects, adults as well as children, inflammatory markers, like C-reactive protein (CRP) correlate with the degree of obesity and insulin resistance and normalise after weight reduction [14]. Despite the overwhelming evidence that low-grade inflammation is associated with diabetes mellitus and atherosclerosis, factors and mechanisms which initiate and uphold low-grade systemic inflammation are still under discussion.

Recently, IgG antibodies against food antigens have been suggested to cause low-grade inflammation in IBS by subtle mucosal inflammation [42]. Food-specific IgG antibodies are able to form an immune complex when bound to food antigens. Such complexes are destroyed by pathogenic cells which leads to the release of proinflammatory cytokines. Aljada *et al* showed that food intake is able to induce significant inflammatory changes, which has been characterised by a decrease in IkappaBalpha and an increase in NF-kappaB binding, plasma C-reactive protein (CRP), and the expression of IKKalpha, IKKbeta as well as p47 (phox) subunit [43]. This state induces a low-grade inflammation condition which may be aggravating for the body. It is documented that individuals with IBS may present with low-grade inflammatory processes in the gut mucosa which is not always connected with a history of a gastrointestinal infection [44]. Therefore, testing IgG against food could be one of the novel diagnostic approaches to identify triggers leading to inflammation in these conditions, and administration of a diet based on the concentration of specific IgG may exert a beneficial effect in patients with IBS and depression [21].

This statement holds as such food elimination therapy based on food-specific IgG antibody testing has been demonstrated to improve the symptoms of a range of conditions of which notably IBS one of them [13]. IgG-mediated food sensitivity may be explained by low level absorption of food macromolecules from the gut [45]. Thus, IgG antibodies to some food components are detectable in healthy individuals although at lower levels. Despite many results confirming the clinical manifestation of elevated levels of IgG antibodies against food antigens, the diagnosis based on their elimination remains controversial. Numerous allergy societies question the usefulness of IgG antibodies tests as a diagnostic method for assessing adverse reactions to food intake [46-48], nevertheless, some do recommend using IgG antibodies tests for research purposes and have in fact been shown to an important tool for investigating food reactions and tolerance [72].

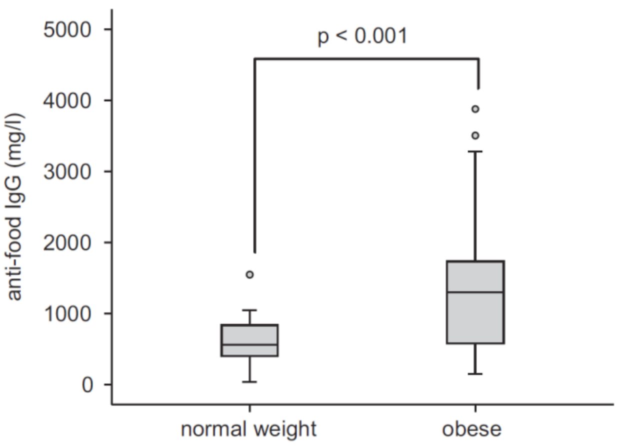


Fig 4. Box and whiskers plot of serum anti-food IgG values in normal weight controls and obese juveniles. Wilders-Truschnig *et al* [14]

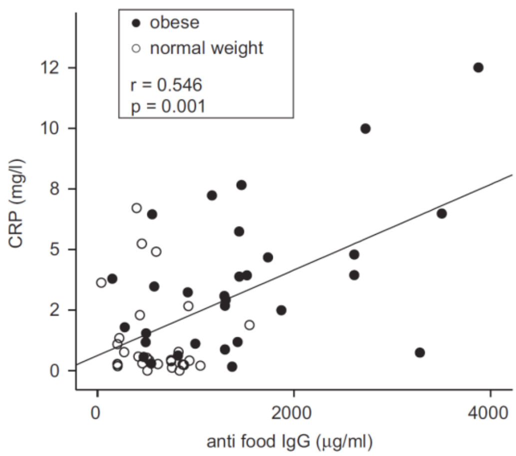


Fig 5. Correlation between IgG antibodies against food antigens and CRP in normal weight (○) and obese (●) juveniles. Wilders-Truschnig *et al* [14]

In a study of obese children by Wilders-Truschnig *et al,* the authors concluded that obese children have significantly higher food-specific IgG antibody values directed against food antigens than normal weight children. Furthermore, they also concluded that such antibodies are tightly associated with low-grade systemic inflammation and with the intima media thickness (IMT) of the common carotid arteries. These findings raise the possibility that food-specific IgG antibodies are pathogenetically involved in the development of obesity and atherosclerosis [14].

The generation of other interleukins (IL-12, IFN) will result in increased IgG antibodies of the IgG1, IgG2 and IgG3 classes [51]. These are pro-inflammatory and are responsible for chronic dietary disorders. Every time a food is consumed, increased levels of IgG1, IgG2 or IgG3 will generate the formation of an immune complex; these complexes will become bound where individual specific imperfections exist in the body. These “activated sites” may be small injuries that had previously become inflamed (e.g. joints), organs damaged by infections (e.g. intestines), or injuries (e.g. to the thyroid) caused by environmental toxins (e.g. mercury) [51]. It is not easy to predict which symptom will appear, because this primarily depends on the patient’s physical condition. If IgG1-IgG3 antibodies are present for regularly consumed foods, the formation of immune complexes leads to chronic inflammation. Non-specific systemic reactions in which low-grade inflammation plays a main role (cardiovascular disease, metabolic syndrome and obesity), have also been linked to the generation of complement activating food-specific IgG antibodies [14, 50].

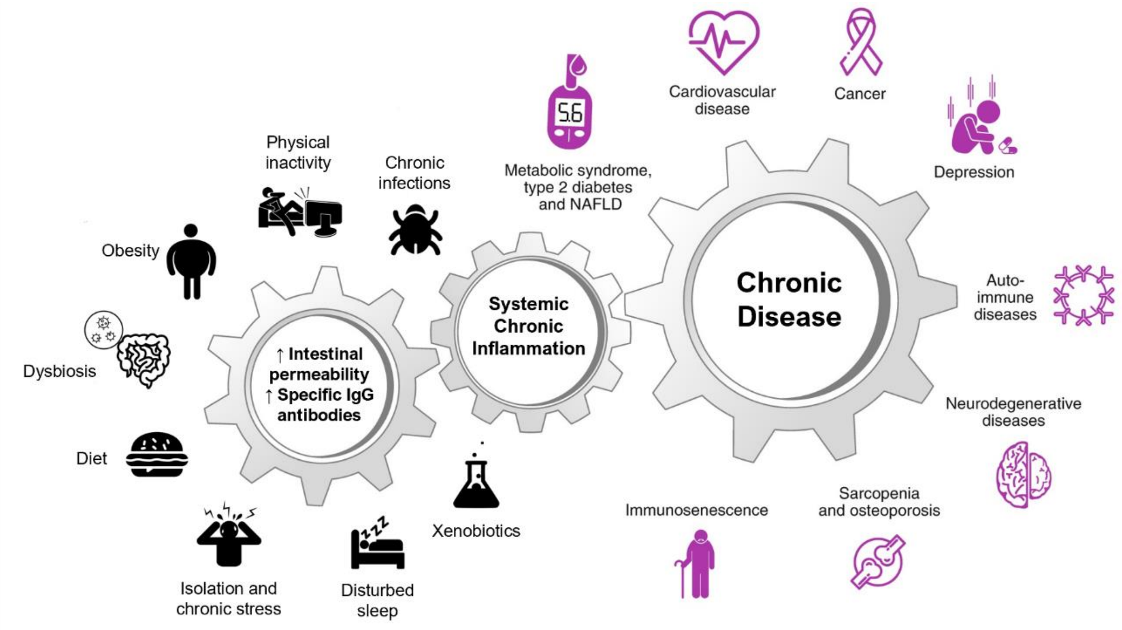
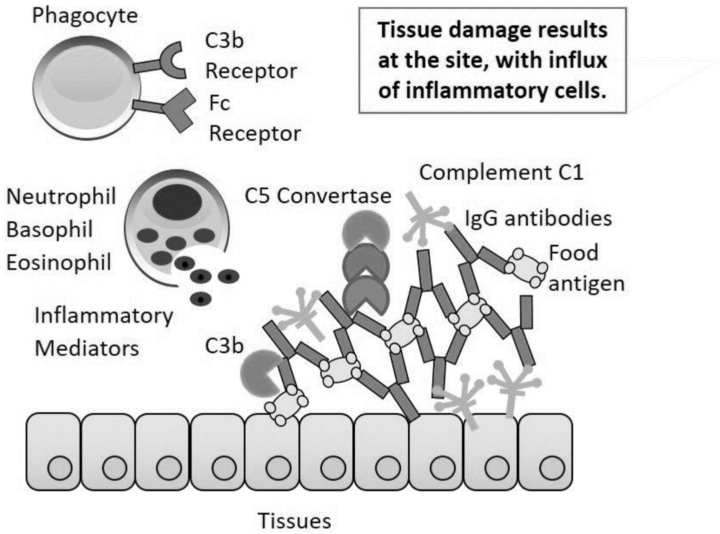


Fig 4. Causes and consequences of low-grade systemic chronic inflammation. Several causes of low-grade systemic chronic inflammation (SCI) and their consequences have been identified.

IgG and complement activation

Any food antigen entering the bloodstream can produce symptoms associated with IgG-mediated food sensitivity. Most food antigens enter the bloodstream through the intestinal epithelium and stimulate the production of specific IgG antibodies. Specific IgG antibodies bind to food antigens that are free in the blood or that have deposited in tissues and form immune complexes [4]. The immune complex activates complement C3 which becomes covalently linked to the IgG forming C3b. Ultimately, the C3b on the immune complex is cleaved forming C3d. During this process, C3a (anaphylatoxin) is released which causes smooth muscle contraction and has a potent vascular effect. Under normal circumstances, circulating C3b binds to the CR1 receptors on red blood cells and are cleared from the circulation in the liver and spleen. Continued production of antibody and formation of immune complexes may result in deposition of immune complexes in tissues which results in activation of the terminal complement pathway C5-9 on the surface of the tissue causing cell lysis and increased inflammation [52, 53].

Tests are beginning to emerge that look for not only food-specific IgG antibodies, but additionally activated complement components particularly that of C3d. C3d is a complement antigen and an activator of the complement cascade system. Reaction to the specified food will worsen if C3d activation is present along with a specific IgG antibody response. The C3 protein attaches to the antigen and amplifies the specific IgG response, increasing the inflammatory potential of IgG titre [54]. However, whilst a single pentameric IgM can initiate the pathway, several, ideally six, IgG molecules are needed. This occurs when C1q binds directly to the surface of the pathogen or food antigen. Since activated C3 components attach to antigens (foods) and not to the circulating specific IgG antibodies, testing for such activated complement components on food-specific IgG antibodies would have limited or no value. The presence of large numbers of circulating immune complexes with food antigens at their core would have to be demonstrated in any test to assess such complement activation [55].



Immunoglobulin G and subclasses

IgG antibodies comprise 70–75% of the immunoglobulins in the serum and are the fundamental antibodies of secondary immune response. Four subclasses of immunoglobulin G are distinguished: the IgG1, IgG2, IgG3, and IgG4 subclasses amount to ~60%, ~32%, ~4%, and ~4% of the IgG antigen pool, respectively. IgG1 and IgG3 have strong pro-inflammatory properties activating the complement pathway. IgG4 on the other hand is a unique molecule that has protective, anti-inflammatory properties and most importantly, does not activate complement and is involved in the generation of tolerance for IgE-mediated reactions [56].

The specific antibodies that are generated primarily depends on which cytokines were formed during the initial exposure. This determines whether a classic food allergy (IgE-mediated) or an asymptomatic food allergy develops, or an immune complex mediated reaction that will later lead to chronic inflammatory processes and thus, to IgG-mediated food sensitivity (IgG1-IgG3-mediated).

Properties of IgG4 and total IgG antibodies

IgG4 is considered to be a non-inflammatory antibody, meaning that it cannot generate chronic inflammatory processes. This is because IgG4 can neither activate the complement nor opsonise the corresponding antigen. However, both processes are required for the generated complex to be recognised and destroyed by phagocytes. Ultimately, this destruction is the inflammatory reaction and is a key why IgG4 does not play a role in IgG-mediated food sensitivity [56].

- An inflammatory reaction cannot occur without opsonisation and complement activation.
- IgG1 and IgG3 have powerful pro-inflammatory properties.
- IgG4 has defensive, anti-inflammatory properties.

PROPERTIES	IgG1	IgG2	IgG3	IgG4
Relative abundance (%)	60	32	4	4
Neutralisation	++	++	++	++
Activation of complement pathway	++	+	+++	
Opsonisation	+++	+	++	
Binding to macrophages	++	+	+++	++
Binding to neutrophils	+		+	

Table 2. Properties of IgG subclasses

IgG4 and food allergy

The genes for antibodies are positioned adjacent to each other on chromosome 14. They are read in succession. Their generation depends on the existing interleukin pattern. Should a majority of IL-4 (interleukin 4) develop, one can assume that increased IgE is being generated and a true Type I allergy is materialising. This is characterised by an immediate reaction after consumption of corresponding foods and may be lethal in the event of anaphylactic shock. IL-4 upregulation occurs in a small fraction of the population (2-6%). This group develops a Type I allergy. All others react to allergen contact (sensitisation) by generating IL-10.
Should a majority of IL-10, which has anti-inflammatory properties, develop upon sensitisation, IgG4 is primarily generated. IgG4 is considered to be a “blocking antibody” to IgE. IgG4 appears in an approximately 10,000 times higher concentration than IgE and can therefore bind to the allergen more quickly and more numerously than IgE can. Because IgG4 only releases approximately 1% of the amount of histamine IgE does, it no longer induces allergic symptoms in nearly all patients. The aim of desensitisation therapy is to trigger the generation of IgG4 to a specific allergen. IgG4 is considered a measure of tolerance to an antigen. The higher the proportion of IgG4/IgE, the greater the effect the desensitisation treatment has had. The presence of specific IgG4 has a positive effect in 95-97% of patients because the antibodies counteract a Type I allergy (the allergens are intercepted by IgG4, so that fewer can react with IgE and cause symptoms). Allergists therefore agree that the significance of IgG4 is for diagnosing and managing IgE-mediated food allergies.

- The IgE/IgG4 ratio is a measure of potential allergic reaction
- High IgE/IgG4: high probability of an allergic reaction
- High IgG4/IgE: low probability of an allergic reaction

The main function of IgG4, appears to be to interfere with and limit immune inflammation induced by complement-fixing antibodies, or, in the case of helminth infection or allergy, by IgE antibodies [56].

The bottom line for allergy diagnosis: IgG4 by itself is unlikely to be a cause of allergic symptoms. In general, the presence of allergen-specific IgG4 indicates that anti-inflammatory, tolerance-inducing mechanisms have been activated. The existence of the IgG4 subclass, it’s up-regulation by anti-inflammatory factors and its own anti-inflammatory characteristics may help the immune system to dampen inappropriate inflammatory reactions [57].

Interpretation of total IgG and IgG4 test results

IgG4 is only detected in total IgG testing in very rare cases (and even then, only medically relevant IgG4 levels) because IgG4 constitutes only 4% one-tenth of the IgG concentration in the blood [57]. As such, mainly IgG1, IgG2 and IgG3 are detected in total IgG testing. In practice, comparative studies have shown that IgG-mediated food sensitivity patients (tested for total IgG) had little or no IgG4 antibodies for corresponding foods. This means that IgG4 diagnostics would not have led to improvement in the conditions of patients with chronic inflammatory problems either.
Because of the potentially high titres of IgG4 reached when an allergy sufferer undergoes desensitisation therapy, there is great potential for cross-reactions to foods. Thus, should someone become desensitised to such an allergen it can be expected that IgG4 titres will be higher.

Clinical relevance for IgG4

Many publications show that IgG4 only has marginal clinical relevance (only in approximately 5% of the population), and few clinical symptoms are elicited through provocation attempts with IgG4-positive foods.
IgG4 is potentially only significant in susceptible young children <18 months old who have not yet developed an IgE response.
IgG4 is not recommended by the majority of the various allergy societies for identifying food allergies or intolerances [46].
It is vitally important to establish the correct clinical utility for the measurement of total IgG and IgG4 specific food antibodies i.e., IgG4 antibodies should not be used to identify IgG-mediated food sensitivity.

IgA and food Sensitivity

IgA is the predominant immunoglobulin secreted by the B cells of the gut. Constituting over 70% of all immunoglobulin present in the intestinal mucosa, where it plays a key role in immune defence via exclusion of potential pathogens and food antigens as a "default" mucosal B cell response. Immunologic responses include local production of secretory IgA (sIgA) antibodies in the intestine; systemic priming with cell-mediated immunity and the generation of antibodies; or tolerance to subsequent antigen challenge [58].

The incidence of IgA deficiency or partial deficiency varies 100-fold among populations. In the United States, an estimated 250,000 individuals have IgA deficiency [59]. In African Americans, the prevalence of IgA deficiency is 1 case per 6000 persons. IgA levels are estimated to be abnormally low in 1:500 subjects, with the incidence as high as 1:100 atopic individuals. Complete absence of IgA is less frequent. A study performed by Weber-Mzell *et al* on 7293 healthy white volunteers demonstrated an IgA deficiency prevalence of 0.21% (definition of IgA deficiency was levels < 0.07g/L) [60]. The same study showed seasonal fluctuations of serum IgA (IgA) concentration; levels of IgA increased in winter. It is argued that IgA deficiency may predispose an individual to IgG-mediated sensitivity as IgA is believed to serve as a barrier to absorption, preventing the uptake of food antigens. In addition, early studies rationalise a systemic decrease in specific IgE and IgG concomitant with a local increase in IgA as an integral role in the induction of oral tolerance. The proposed mechanism was thought to be due to the influence of Th2 cytokines and TGF-beta, which act to suppress IgG/IgE B cell differentiation, but at the same time enhance

IgA B cell differentiation. In other words, oral tolerance was believed to be associated with concomitant local IgA immunity.
IgA is produced in response to food antigens which cross the gut epithelium. IgA is synthesised in the lymph nodes and secreted directly into the gut lumen and blood stream. Generally, IgA reactions are delayed reactions which may take several days to manifest after consuming a particular food. Elevated IgA levels are believed to indicate that there is damage to the intestinal lining leading to increased intestinal permeability. IgA antibodies to specific foods may form when the lining of the intestinal tract becomes inflamed or damaged due to stress, alcohol, medications or other inflammation-causing conditions. Patients with suspected IgG-mediated food sensitivity or having increased intestinal permeability, colitis, coeliac disease, or irritable bowel syndrome (IBS) will produce IgA antibodies against dietary antigens and may benefit from testing in parallel with IgG as there is some evidence that selected patients may be negative for IgG against a specific food but positive for IgA or vice versa [78].

Food antigens raw vs cooked

Despite the first documented case of food allergy to cooked food in 1921 by Prausnitz and Kustner [61], the majority of food antigens used in commercial tests commercial food antigens are prepared from raw food. Processed foods and their ingredients are subjected to a variety of conditions, which may cause alterations in immunodominant epitopes, potentially affecting antigenic properties.
The different types of food processing include thermal as well as non-thermal treatments, and each type of process may have a different effect on epitopes. Processing may affect foods in a manner that could potentially induce the masking or unmasking of antigenic epitopes, thereby enhancing or reducing antigen recognition and potentially altering the antigenicity of the offending food [62].

Limited studies have suggested that the determination of allergy and IgG-mediated food sensitivity to food in the population could be improved by measuring IgE, IgG, IgA and IgM antibodies against both raw and processed food antigens [79]. Further research is needed to examine just how much an elimination diet, based on the results of such testing, could contribute to the reduction of autoantibodies seen in test subjects and a concomitant improvement in the conditions of those suffering from autoimmunity, metabolic syndrome, aging and neurodegenerative disorders.

IgG and food additives & preservatives

There are thousands of substances added to various foods for the purposes of colouring, flavouring and preserving. Additives are usually only a very small component of foods but have been suspected of causing various reactions. Food additives include the following groups:

- Food dyes and colourings (such as tartrazine, annatto and carmine)
- Antioxidants (such as BHA and BHT)
- Emulsifiers and stabilizers (such as gums and lecithin)
- Flavourings and taste enhancers (such as MSG, spices and sweeteners)
- Preservatives (such as benzoates, nitrates and sulphites)

Food additive intolerance is a non-IgE-mediated food hypersensitivity. Recent studies have demonstrated that in many cases the hypersensitivity is induced by the food additives via a direct mast cell activation known as a pseudoallergic reaction, although the exact pathophysiology is unknown [63]. Adverse effects due to various pharmacological or other mechanisms have also been documented and may be more common. Reactions are usually dose-related however individual tolerance may be decreased due to a combination of genetic and environment factors one reason or another and may fluctuate from time to time.
For synthetic substances, in 98% of cases, the response is a pseudoallergic reaction. The clinical symptoms of these reactions mimic an IgE-mediated allergic immediate type reaction but without the involvement of IgE [63]. Synthetic food additives consist of small chemical molecules that will not in themselves generate an antibody response. Therefore, tests for food-specific IgE and IgG are of no clinical value if an adverse reaction is suspected against these additives. Additives of plant or animal origin have been shown to generate an antibody response and are therefore capable of generating an IgE-mediated allergic response or IgG-mediated food sensitivity.

Effects of Elimination Diet

According to Isolauri *et al*, an elimination diet based on the results of the measurement of food-specific IgG antibody levels may be equally beneficial in terms of symptom relief as is the case with IgE-dependent allergy [64]. A study conducted by the University of York on behalf of the British Allergy Foundation in 2001 investigated the usefulness of an elimination diet used after determination of serum levels of food-specific IgG antibodies. Of the 1761 subjects used in the final statistical analysis, as many as 50% of the subjects observed a considerable improvement of health after introduction of the elimination diet based on their food-specific IgG testing results and 70% reported health benefits [65]. This study is suggestive enough to justify the need for investigating the potential contribution of IgG-mediated food sensitivity to many disease entities.

The potential role of IgG-mediated food sensitivity in the pathogenesis of IBS is being extensively investigated. IBS is a chronic functional disorder of the intestines manifested by frequent abdominal pain, bloating and constipation and/or diarrhoea, which may occur alternately. The prevalence of IBS in the general population is high and is estimated at 12–22% [66]. Given the heterogeneity of the abnormalities and the multifactorial aetiology of IBS, the involvement of IgG-mediated food sensitivity in the initiation of the pathological changes seems likely. Interestingly, most patients suffering from functional disorders of the gastrointestinal tract report that certain foods exacerbate their symptoms [67]. The management of IBS focuses mainly on administration of antispasmodic drugs, drugs that modify intestinal function, antibiotics, antidepressants and analgesics. Atkinson *et al* [13] showed that an elimination diet can be effective in relieving the symptoms of IBS. After 12 weeks of the diet a 10% improvement in well-being and resolution of the symptoms were observed ($p = 0.024$). The quality of life also improved. Notably, in patients who decreased the restrictiveness of the diet, a 24% worsening of the symptoms was observed compared to patients strictly adhering to the dietary guidelines. Drisco *et al* [66] conducted a study in 20 patients meeting the Rome II criteria for IBS. The patients followed a diet for 6 months that was based on the results of the tests for IgG-mediated food sensitivity. The patients were also instructed to use a probiotic. The study showed abnormal titres of IgG antibodies specific for selected food components in all the patients. Using a diet based on the results of testing for food-specific IgG antibodies resulted in a statistically significant improvement in symptoms (improved stool frequency, pain relief) ($p = 0.05$) and the quality of life ($p = 0.0001$). The further step of the analysis involved an open extension. The patients adhering to the diet reported considerable improvement that was greater than the improvement observed with the intake of a probiotic. Also, other researchers have demonstrated the utility of introducing a diet based on measurements of the levels of IgG antibodies to food antigens in patients with IBS [67, 68].

The current clinical guidelines of the American College of Gastroenterology for the management of IBS, recommends a limited trial of a low fermentable oligosaccharides, disaccharides, monosaccharides, polyols (FODMAP) diet in patients with IBS to improve global symptoms. However, they also advise that 'multiple tests are marketed to diagnose food sensitivities; however, none have been validated, and most have not been subjected to rigorous, blinded trials. And as such serum IgG panels have not been validated and cannot be recommended at present [81]. In a resent published study by *Ostrowska et al* [82] the authors compared the effectiveness of three different diet plans in treating patients with mixed IBS:

- G1-FM-low-FODMAP diet
- G2-IP-IgG-based elimination-rotation-diet
- G3-K - Control group, classic diet recommended by an attending gastroenterologist

Significant differences in reduction of IBS symptoms were found between the 3 groups.
All IBS symptoms as well as comorbid symptoms like headache, skin conditions, constant tiredness/weakness significantly improved or disappeared completely in the G2-IP group ($p<0.008$). While in G1-FM group only some IBS symptoms like bloating, gastric fullness significantly improved. In group G3-K no significant improvement was seen. Their conclusion was that the most effective diet in the treatment of patients with mixed IBS was the elimination-rotation diet based on IgG-dependent food hypersensitivity test. This study shows that a personalised dietary approach is more effective in treating IBS-M than generalised diet recommendations. Only the IgG elimination-rotation diet could demonstrate significant improvements in all of the monitored IBS-M symptoms as well as extra-intestinal symptoms. One possible strategy could be to start with the elimination-rotation diet, as it was proven to be the more effective diet in this open study, and in cases of persistent symptoms, it could be combined with a low-FODMAP diet.

The therapeutic application of a diet based on the results of the tests assessing IgG-mediated food sensitivity has also been shown in patients with Crohn's disease [18]. The authors of the analysis, given the multifactorial aetiology of the disease, suggested a potential contribution of the immune response to food antigens to the maintenance of inflammation. For this reason, a pilot study was conducted in 79 adult patients with Crohn's disease. The control group consisted of 20 healthy volunteers. The study showed markedly higher serum levels of food-specific IgG in patients with organic bowel disease compared to the control group. IgG antibodies to cheese and to baker's yeast were demonstrated in 84% and 83% of the patients, respectively. Following a diet based on the results of the measurement of specific IgG for food antigens considerably improved stool frequency, pain and patients' well-being. Decreased secretion of interferon gamma (IFN-γ) by T cells was also observed. The levels of the Eosinophil-derived neurotoxin (EDN protein) in the stool did not, however, change. The findings of the study demonstrate that implementation of a diet based on testing for IgG-mediated food sensitivity in patients with Crohn's disease is justified. However, this is the first analysis of this type and therefore drawing any binding conclusions requires further dietary studies in a group of patients with inflammatory bowel disease.

However, there is a necessity to carefully select research tools, i.e., tests that are based on reliable methods and that assess the correct parameter [67].

The best tools for the assessment of IgG-mediated food sensitivity are assays that assess all the IgG subclasses (IgG1–IgG4) quantitatively (ELISA). There are currently many commercially available services offering testing for IgG food antibodies. These include capillary blood and dried blood spot collections from a finger prick, in addition to standard venous serum testing. Such assays are often performed using assays developed 'in house' or 'home brew' tests, and often lack appropriate levels of validation. It is important that all such assays for food-specific IgG antibodies should conform to appropriate quality standards and diagnostic kits used for such assays should be appropriately CE marked. There is a need for more standardisation of these assays and some laboratories offer an external quality assessment scheme for users in order to achieve this. For this reason, the selection of an appropriate test to assess the levels of IgG antibodies to food components should be a priority.

Evidence-based clinical relevance of food specific serum IgG antibodies

A growing body of medical literature supports the clinical value of measuring food-specific IgG antibodies to guide therapeutic dietary changes. For example, a number of studies involve IBS patients. In all studies, significant clinical improvement was gained by using IgG (total) testing to screen for foods for dietary exclusion [1, 13, 66, 69, 70].

IBS is estimated to occur in 12% to 22% of the UK population and is a disorder of high direct and indirect medical costs [71]. Any improved treatment and management would be of significant benefit not only to patient outcome, but also to the reduction in health care costs. In a review of all published literature from 1966 to 2015 relating to IBS, a report in the World Journal of Gastroenterology in 2015 [40], concluded that hypersensitivity reactions may play a role in causing IBS symptoms in a subset of patients. Furthermore, the increase of food-specific IgG titres could be a specific reaction, rather than a non-specific response to increased gut mucosal permeability. The authors concluded that 'Pending further scientific evidence, the concept of adverse food reactions should be included as a possible cause of IBS, and a dietary approach may have a place in the routine clinical management of IBS'.
A number of other studies looking at a variety of conditions showed food-specific IgG antibody testing to be clinically useful in ameliorating symptoms [2, 14, 16].

Discussion

Despite the large volume of published works implicating IgG-mediated food sensitivity as part of the aetiology of complex diseases, the clinical utility of food-specific IgG antibody measurements has been called into question.

The efficacy of a diet based on the measurement of IgG antibodies specific for food components has been demonstrated for a number of disease entities. Excellent results have been obtained in patients with migraine, respiratory disease, IBS, obesity and IBD [14, 16, 17, 18, 40]. A diet based on testing for food-specific IgG antibodies has shown to be an alternative and safe treatment for patients with chronic conditions [65]. It is important to remember that such testing has been performed by many reputable laboratories all over the world for many years resulting in many hundreds of thousands of tests, that in a great many cases have resulted in relief for the patient from persistent symptoms [11, 13-21].

The notion that such testing merely reflects a normal immune response and has no clinical value, is at odds with the increasing large body of published studies. With the large body of evidence that has now been accumulated and with the recent evidence demonstrating the crucial role that IgG class antibodies play in initiating tolerance [72], it is time for a balanced medical discussion about what such testing can be used for and its limitations. The determination of serum food-specific IgG antibodies potentially opens up new diagnostic and therapeutic pathways for patients who are hypersensitive to food components, which can be alleviated by food elimination diets based on food-specific IgG antibody levels, for a variety of complex and challenging conditions.

Key Definitions

The nomenclature surrounding allergy and intolerance is varied and confusing. Often terms are used interchangeably and sometimes incorrectly, this is particularly true for IgG-mediated reactions.

Food Specific IgG Antibody Testing:
Also called, IgG-Mediated Food Hypersensitivity, IgG-Mediated Allergy, IgG Food Sensitivity, IgG-Mediated Reactions, IgG-Mediated Food Reactions, IgG Food Intolerance, IgG-mediated food sensitivity, IgG-Dependent Hypersensitivity, Food Intolerance, Food Sensitivity, Food-Specific IgG Antibody Testing, Food IgG Testing, Food Hypersensitivity, Food IgG Antibody, Food Sensitivity Reactions, Chronic Food Sensitivity, Chronic IgG-Mediated Reactions, Type III Allergy, Type III Hypersensitivity, Specific IgG Antibody Testing, Non-Immediate (Delayed) IgG Responses, Delayed Reactions, Classic Food Intolerance.

Food Intolerance - Non-immune mediated reactions or food intolerances include metabolic, pharmacologic, toxic, and undefined mechanisms. In some cases, these may mimic reactions typical of an immunologic response.

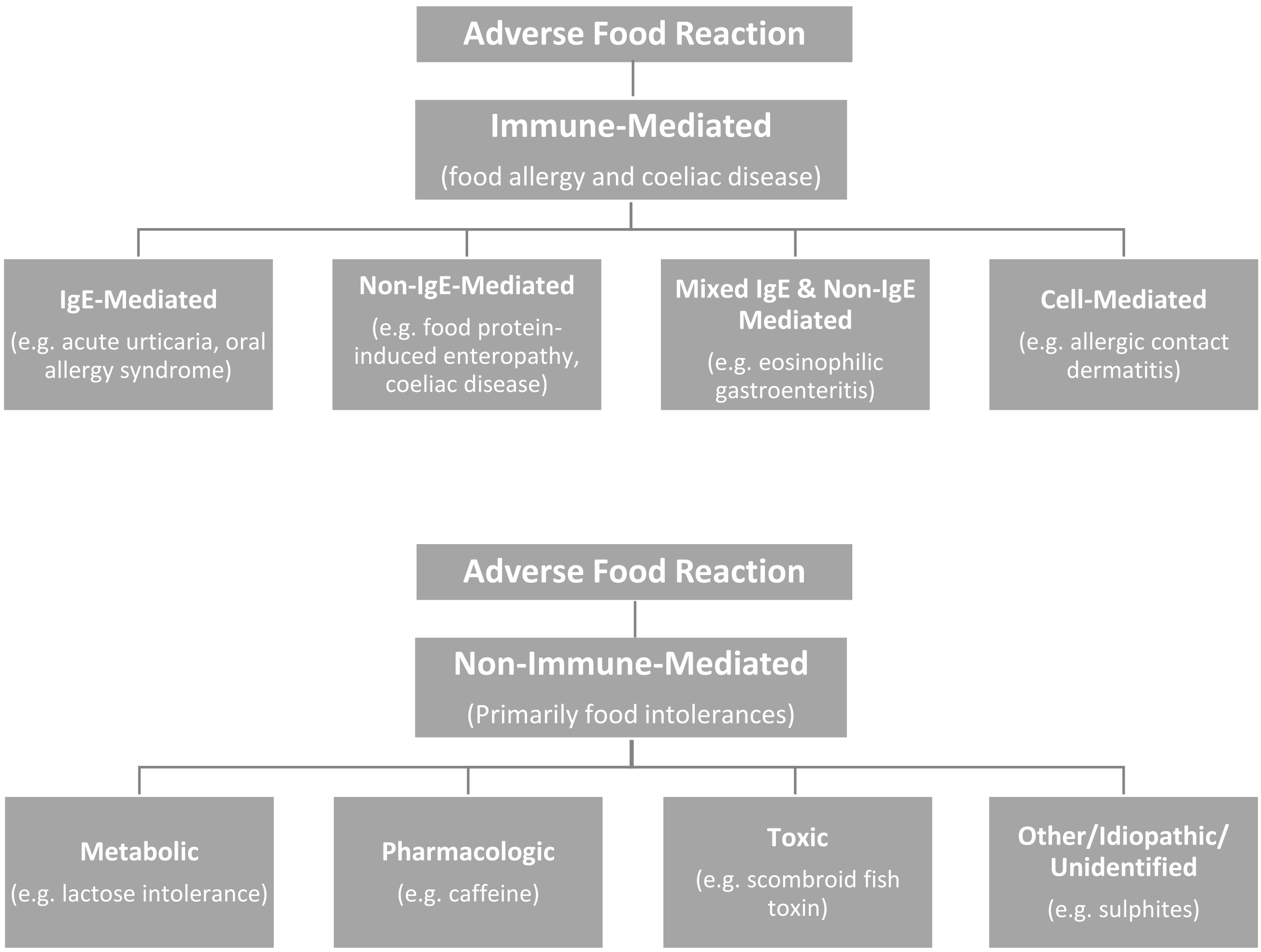


Fig 1. Types of adverse reactions to food [76].

Non-Immune-Mediated Reactions			
Implicated Substance		**Associated Foods**	**Possible Symptoms**
Enzymes	lactase deficiency	lactose in dairy products	nausea, diarrhoea, gas, bloating, and abdominal cramps
	alpha-galactosidase insufficiency	cruciferous vegetables, legumes	gas, bloating
Chemicals	histamine	fish, sauerkraut, cheese	headaches, rashes, itching, diarrhoea, vomiting and/or abdominal pain
	methylxanthine	cola, coffee, chocolate, tea	anxiety, panic disorders
	tyramine	cheese, pickled herring	headache, palpitations, nausea, vomiting
	tryptamine	fermented foods (soy sauce), Acacia species (incl. beans)	restlessness, agitation, gastrointestinal distress, muscle tension, may be hallucinogenic
Toxins	aflatoxin	peanuts, cereal grains	chronic exposure: liver disease, increased risk of liver cancer
	saxitoxin	shellfish	inhalation: numbness & tingling of lips, tongue, and fingertips, followed by numbness of the neck and extremities and motor incoordination. Other symptoms may include light-headedness, dizziness, weakness, confusion, memory loss, and headache
	ergot	cereal grains	numbness, tingling & burning in limbs, feeble pulse, restlessness, stupor, delirium
	cyanogenic glycosides	cassava, stone fruits (e.g. peach, apricot)	chronic exposure may lead to thyroid and neurological disorders

Table 1. Non-Immune-Mediated Reactions

***IgG-mediated food sensitivity* -** Whereas food allergy is typically mediated by IgE antibodies, food intolerance can be mediated by IgG class antibodies. The prevalence of food intolerance is believed to be as high as 45% of the UK population according to figures produced by Allergy UK 2009. The Association of UK Dietitians (BDA), 2015 reported that as many as 20% of the population experience some reactions to foods which make them believe they do have a food hypersensitivity; however, the true prevalence of food intolerance remains unknown due to insufficient data. IgG-mediated food sensitivity is believed to be caused by increased gut permeability, which permits food proteins to gain access to the circulation and trigger food-specific IgG production. Increased production of food-specific IgG antibodies has been linked with increased levels of low-grade inflammation and the production of immune complexes.

IgE-Mediated Food Allergy:

Allergy is a hypersensitivity reaction initiated by immunological mechanisms. Allergy can be antibody- or cell-mediated. In the majority of cases the antibody typically responsible for an allergic reaction belongs to the IgE isotype and these individuals may be referred to as suffering from an IgE-mediated allergy. Not all IgE associated 'allergic' reactions occur in 'atopic' subjects, where the genetics of an individual's immune system has a tendency towards producing IgE class antibodies. IgE-mediated food allergies are a cause of particular concern in young children, where the incidence of food allergy (often life threatening) is estimated to be greater in children (5-8%) than in adults (1-2%) [73].

Pseudoallergy:
Also called: Pseudoallergy (Intolerance), non-allergic hypersensitivity

Pseudoallergy mimics immediate-type allergic reactions clinically without evidence of underlying immunological mechanisms. The most common triggers of pseudoallergic reactions are aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), as well as some food ingredients and additives, such as salicylates, benzoates, and tartrazine. These reactions do not involve IgE sensitisation rather they are the result of direct activation of mast cells/basophils or eosinophils and can, therefore, occur on first exposure. Pseudoallergic reactions are dose-dependent and usually occur with chemically non-related substances. The diagnosis is difficult because skin tests and serology are uninformative. Diagnosis of non-allergic hypersensitivity is based on a distinctive clinical pattern, time course, clinical signs, response to elimination of the cause and by the use of specialist basophil activation tests.

Antibodies:
The immune system makes five major antibodies, which are known by different letters: G, E, A, M and D. The G type, called IgG, is the most common. Of all antibodies found in blood, 80% are IgG. IgG antibody reactions are considered to be involved in type III hypersensitivity often referred to as IgG-mediated food sensitivity or food sensitivity. There are four subclasses of IgG: IgG1, IgG2, IgG3 and IgG4. All subclasses activate the classical complement pathway except IgG4.

Food Antigens:
Defined as those specific components of food or ingredients within food (typically proteins, but sometimes also chemical haptens) that are recognised by antigen-specific immune cells and elicit specific immunologic reactions, resulting in characteristic symptoms. Some antigens (most often from fruits and vegetables) cause reactions primarily if eaten when raw. However, most food antigens can still cause reactions even after they have been cooked or have undergone digestion in the stomach and intestines.
Food oils—such as soy, corn, peanut, and sesame—range from very low antigenicity (if virtually all of the food protein is removed in processing) to very high antigenicity (if little of the food protein is removed in processing).

Antigen / Antibody Interaction:
Also called: Antigen-Antibody Reaction

Antigen-antibody interaction is a specific chemical interaction between antibodies produced by B cells and antigens during immune reactions. The antigens and antibodies combine by a process called agglutination. It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. There are several types of antibodies and antigens, and each antibody is capable of binding only to a specific antigen. The specificity of the binding is due to specific chemical constitution of each antibody. The antigenic determinant or epitope is recognised by the paratope of the antibody, situated at the variable region of the polypeptide chain. The variable region in turn has hyper-variable regions which are unique amino acid sequences in each antibody. Antigens are bound to antibodies through weak and non-covalent interactions such as electrostatic interactions, hydrogen bonds, Van der Waals forces, and hydrophobic interactions.

Food Antigen Alteration:
The form of antigen being tested is not the same as what the patient reacts to. For example, whey protein is altered by high heat, so someone with a whey allergy may have no reaction to heat-altered milk products. Similarly, raw eggs may show no reaction, but cooked eggs may be a problem.

Cross-Reactions:
A phenomenon called cross-reactivity may occur when an antibody reacts not only with the original antigen, but also with a similar antigen. Cross-reactivity occurs when a food antigen shares structural or sequence similarity

with a different food antigen or aeroallergen (airborne substances, (e.g. pollen, spores etc.) that trigger hypersensitivity reactions), which may then trigger an adverse reaction similar to that triggered by the original food antigen.

Tolerance:
Also called: Immune tolerance, or immunological tolerance, or immunotolerance.

Tolerance is the prevention of an immune response against a particular antigen. For instance, the immune system is generally tolerant of self-antigens, so it does not usually attack the body's own cells, tissues, and organs. However, when tolerance is lost, disorders like autoimmune disease, food allergy or IgG-mediated food sensitivity may occur.

Increased Intestinal Permeability:
Also called: Leaky Gut Syndrome, enhanced permeability, hyper permeability.

Leaky gut syndrome is caused by inflammation in the gut lining. Inflammation can be caused by a variety of different triggers, including food allergies or sensitivities, abnormal gut flora, stress, certain drugs, and alcohol. When the gut lining is inflamed, food particles can leak through and be considered foreign bodies. In the case of IgG-mediated food sensitivity, leaky gut syndrome can lead to elevated IgG levels to some of the foods a person normally eats resulting in the formation of immune complexes, which can then trigger inflammation and symptoms.

Inflammation:
Circulating IgG antibodies form immune complexes with antigen. This is considered a Type III delayed hypersensitivity reaction, and typically occurs over several hours to several days. The release of IgG antibodies to specific foods is considered normal, as is the formation of antigen-antibody complexes (which form when a food antigen meets an IgG antibody and they bind together). Formation of the complexes activates the complement pathway and releases inflammatory mediators.

However, the IgG-Ag immune complexes are usually cleared by macrophages but, in the presence of excess antigen, macrophages may saturate their capacity to remove immune complexes, causing the excess to be deposited in tissue. Deposition of IgG-Ag complexes causes inflammation and tissue damage, which may contribute to specific health issues. Inflammation will continue to be triggered if the reactive food remains a regular part of the diet since more immune complexes will form, and as such contribute to a variety of symptoms.

Complement:
Also called: complement cascade

The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promote inflammation, and attack the pathogen's cell membrane or other activating antigens such as food. It is part of the innate immune system, which is not adaptable and does not change during an individual's lifetime. The complement system can, however, be recruited and brought into action by antibodies generated by the adaptive immune system. Over 30 proteins and protein fragments make up the complement system, including serum proteins, and cell membrane receptors. They account for about 10% of the globulin fraction of blood serum. Three biochemical pathways activate the complement system: the classical complement pathway, the alternative complement pathway, and the lectin pathway.

Opsonising: An antigen must first be marked (and thus made visible) so that phagocytes, or macrophages, can identify it. This takes place through antibodies. IgG1 and IgG3 are the IgG antibodies most capable of this, whereas IgG4 possesses no opsonising action. This means that IgG4 cannot appropriately mark the antigens in order to be recognised by phagocytes.

Irritable Bowel Syndrome (IBS):
Also called spastic colitis, mucus colitis, nervous colon syndrome.

A common gastrointestinal disorder, which rather than a disease entity, involves a collection of symptoms including an abnormal condition of gut contractions (motility) and increased gut sensations (visceral hypersensitivity) characterised by abdominal pain / discomfort, gas, bloating, mucous in stools, and irregular bowel habits with constipation or diarrhoea or alternating diarrhoea and constipation. Symptoms tend to be chronic, intermittent and to wax and wane over the years. Although IBS can cause chronic recurrent abdominal discomfort, it does not lead to any serious abdominal organ problems. Making the diagnosis is usually done by suspecting the condition and confirming IBS by excluding other causes of abdominal illnesses. Treatment is directed toward relief of symptoms and includes changes in diet (eating a moderate high fibre and avoiding caffeine, milk products and sweeteners), exercise, relaxation techniques, and medications. There are a number of publications [1, 13, 40, 66] that have shown efficacy for an exclusion diet based on food-specific IgG antibody testing.

Inflammatory Bowel Disease (IBD):
Inflammatory bowel disease (IBD) is an umbrella term used to describe disorders that involve chronic inflammation of the digestive tract. Types of IBD include:

- **Ulcerative colitis.** This condition causes long-lasting inflammation and sores (ulcers) in the innermost lining of the large intestine (colon) and rectum.
- **Crohn's disease.** This type of IBD is characterised by inflammation of the lining of the digestive tract, which often spreads deep into affected tissues.

Both ulcerative colitis and Crohn's disease usually involve severe diarrhoea, abdominal pain, fatigue and weight loss. IBD can be debilitating and sometimes leads to life-threatening complications.
The exact cause of inflammatory bowel disease remains unknown. Previously it has been suggested that diet and stress were implicated, but now it is believed that these factors may aggravate the conditions rather than cause IBD.

Migraine:
Also called: Chronic Headache

Migraine is characterized by recurrent unilateral headache usually accompanied by nausea/vomiting, photophobia, and/or phonophobia, with 1-year- period prevalence of 10-12% in adults, 6% among men, and 15-18% in women [74]. The mechanisms underlying primary migraine are still unknown. Besides different genetic mutations, there is evidence of a profound role of meningeal inflammation, and environmental trigger factors are thought to play an important role. Sensitivity to a particular food or foods is one of the most common triggers of migraine. IgG antibodies against various food antigens have been reported to be associated with migraine. There are a number of publications [15, 16, 74] that have shown efficacy for an exclusion diet based on food-specific IgG antibody testing in demonstrating a decrease in headache attacks and significant improvement in symptoms.

Chronic Disease:
A disease that persists for a long time. A chronic disease is one lasting 3 months or more, by the definition of the U.S. National Center for Health Statistics. Chronic diseases generally cannot be prevented by vaccines or cured by medication, nor do they just disappear. In the UK, more than 15 million people are currently living with at least one chronic condition and this number is expected to continue to rise [75]. Health damaging behaviours - particularly tobacco use, lack of physical activity, and poor eating habits - are major contributors to the leading chronic diseases.

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