Allergic reactions to foods may be classified as either IgE-mediated or non-IgE-mediated - the role of the former in food allergy being well established. However, interestingly enough, the majority of food allergies are associated with specific non-IgE-mediated immune sensitivities. As such, appropriate tests must be utilized to identify possible causes including food-antigen specific IgG antibodies. There are many testing methods available for the detection of food allergies including the skin prick test and RAST, or radioallergosorbent test. Unfortunately, both of these methods only assess for allergen-specific IgE antibodies from the patient’s serum. This poses considerable limitations in the clinical assessment of the chronically unwell patient.

The Skin Prick Test – Pitfalls

With regards to IgE testing, the ELISA method offers an excellent confirmatory test for IgE-mediated food allergies when skin prick testing is equivocal or negative, as it is not unusual for a patient to be skin prick test negative and ELISA positive. Generally, the assumption in such a case is that either the extracts used for IgE skin prick testing were defective, unstable or non-standardized. Conversely, a false positive skin test may be due to nonspecific enhancement of the hypersensitive reaction through an axon reflex of a neighboring strong wheal-and-flare reaction. In addition, skin prick testing does pose a health risk to the patient, as eluates of protein extracts are pierced through the skin. Anaphylaxis is a possibility and resuscitation equipment must be on hand. Furthermore, skin prick testing does not assess for delayed-onset food allergies mediated through IgG antibodies. IgG concentrations increase from repeated exposure to food antigens. IgG-mediated food reactions occur hours to days after exposure to the incriminating foods, and unlike that of IgE, IgG related symptoms are cumulative in nature.

Since the discovery of IgE in 1967, conventional medical practice has focused chiefly on IgE-mediated allergies as identified primarily through skin prick testing. As our understanding of the disease model progresses with physiological mechanisms finding root in regulation of oral tolerance, the clinical importance of IgG antibodies is rapidly following suit as a key player in the allergic model of disease and chronic pathology.

The IgG Immunoglobulin Class

The IgG immunoglobulin class has an exceptionally long half-life in circulation and makes up about 75% of the total serum immunoglobulin pool. This class is comprised of four known subtypes: IgG1, IgG2, IgG3, and IgG4. IgG1 constitutes about 68% of total IgG; IgG2, 20%; IgG3, 8%; and IgG4, 4%. IgG1 promote the release of histamine and other inflammatory mediators from these immune cells. The result is a wheal-and-flare reaction marked by redness and swelling. However, identification of such mast cell dependent reactions for the detection of food allergies does have its pitfalls in addition to those mentioned. First, diseases such as eczema may attenuate the skin response. Second, there is decreased reactivity of the skin in infants and elderly patients making this testing method inappropriate for these populations. In addition, mast cells from different sites of the body (skin, lungs, and gastrointestinal tract) exhibit marked heterogeneity with respect to their functional properties. This is of fundamental importance from a clinical perspective since one cannot simply extrapolate the results from a skin prick test and assume a direct correlation to that which is occurring in the gut. Furthermore, skin prick testing does not assess for delayed-onset food allergies mediated through IgG antibodies. IgG-related symptoms are cumulative in nature.
through IgG3 are capable of binding complement and initiating complement-mediated tissue injury, whereas IgG4 is not. However, it is argued that altered IgG4 through immune complex formation may act as an autoantigen. Since IgG levels increase with exposure, these complexes may reach appreciable levels over time. Autoantibodies such as of the IgM class, formed to these altered IgG4 autoantigens, may cross-link cell-bound IgG4 and activate complement. Such a mechanism has been reported responsible for the exacerbation of symptoms in atopic eczema patients where high-molecular-weight (i.e. 21S or more) immune complexes have been identified. An autoimmune process such as this clearly deserves considerable attention to its clinical implications in chronic allergic disease.

Interesting among the IgG class of antibodies are the IgG receptors, FcγR. Since IgG represents the dominant antibody class in plasma, receptors for IgG have been intensively studied over the years. These receptors are found on a wide variety of immune cells and are said to serve as a bridge between the cellular and humoral parts of the immune system. Effector functions that can be triggered by FcγR include: antibody-dependent cellular cytotoxicity (ADCC), antigen presentation, cytokine release, phagocytosis, degranulation, and regulation of antibody production. With a constant stream of IgG antibodies in circulation due to chronic challenge, inappropriate regulation of FcγR-mediated responses, or inefficient FcγR function may lead to a hyperresponsive state with greatly magnified effector responses that may subsequently promote inflammatory disease and increase susceptibility to autoimmunity.

**The Gut Immune System**

It is well known that a significant portion of ingested proteins from the food stream reach the gut-associated lymphoid tissues (GALT), in an immunologically intact form capable of stimulating immune responses in the susceptible individual. This susceptibility rests in the competency of the immunoregulatory mechanisms of the GALT that normally prevent the induction of a hypersensitive response to otherwise innocuous food challenges. It is undesirable to be intolerant to the foods we eat. Mobilization of the GALT against food antigens defines loss of oral tolerance to foods, and may provoke injurious local and systemic immune responses. The gut mucosal response, particularly that involving an antibody response, is highly dependent on T cell help. T helper pattern induced clonal expansion may proceed through a cell-mediated (Th1) response, humoral (Th2) response, or immune tolerance (Th3). It is important to note that T cells of the mucosal lymphoid tissue are heavily biased toward a Th2 response. This accounts for the predominance of the protective IgA isotype in mucosal effector tissues. However, the phenotypic polarization of immunoglobulin producing B cells favoring IgA is heavily influenced by the cytokine profile present in the mucosal milieu. Interleukin-4 (IL-4) for example, promotes isotype switching to both IgG1 subclass and IgE whereas, the cytokine transforming growth factor beta (TGF-β) favors B cell class switching from IgG and IgE to IgA thereby, suppressing any potential for a Th1 or Th2 inflammatory response to dietary antigen.

Ideally the intestinal immune system can discriminate proteins in the food stream as innocuous and not of any pathogenic importance. It can be said in this case that a state of tolerance is achieved with suppression of IgE and IgG responses, and enhancement of a local secretory IgA antibody response. Certainly, the integrity of the mucosal barrier with its immune constituents in competent interplay is prerequisite for oral tolerance induction, and susceptibility to breakdown of oral tolerance varies individually. Loss of mucosal barrier integrity and genetic polymorphisms in markers of innate immunity, including that of the FcγR class of IgG receptors, no doubt play a key role in abrogation of oral tolerance to dietary challenge. The biological mechanisms of food allergies are diverse and remain to be explored. Loss of tolerance, as exemplified by elevated food-specific IgG class antibodies is a breakdown of the GALT to distinguish antigens of nonpathogenic importance, abrogating the metabolic usefulness of the foods we eat, and instigating the potential for inflammatory and autoimmune conditions.

Effective assessment of food allergies, especially through IgG testing should be as routine for the practitioner as ordering a CBC (Complete Blood Count). Identification of elevated food-specific IgG antibodies is a means to identify loss of tolerance to dietary proteins, and provides the practitioner with a tool to direct care in the appropriate manner. Once identified, treatment of food allergies includes dietary rotation of

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compatible foods and avoidance of allergenic ones, in addition to cogent measures to reestablish tolerance.

**The ELISA Method**

The ELISA colorimetric technique, or Enzyme-Linked Immunosorbent Assay, is a useful screen for immediate and delayed food allergies mediated through immunoglobulin E (IgE) and immunoglobulin G (IgG), respectively. Allergic reactions to foods are characterized by elevated allergen-specific immunoglobulin serum levels with activation of immune mediators of inflammation. Food allergies are implicated in intestinal pathology, as typified by celiac disease, and a number of systemic inflammatory conditions.21,22

ELISA is a quantitative/semiquantitative in vitro analysis designed to detect and quantify IgG and IgE antibodies reactive to various food proteins. Through the ELISA testing method, lyophilized food proteins are immobilized by adsorption to plastic wells and reacted with the serum portion of the individual’s blood sample. After washing, the plate is reacted with an HRP- labeled anti-human IgG or IgE antibody conjugate. The enzyme tag, HRP, or horseradish peroxidase, facilitates a color change upon addition of its substrate, a chromagen, to allow for easy detection of antigen-antibody interaction within the wells. The intensity of the color change is quantified through spectrophotometric analysis, and is proportional to the concentration of food antigen-specific IgG or IgE antibodies present in the serum sample.

**The ELISA Method – Reproducible, Reliable and Valid**

There are several industry standards that should be considered for ELISA testing to allow its implementation as a routine method suitable for analysis of food allergies. Official criteria for any bioanalytical method includes clear demonstration of reproducibility and reliability for its intended use based on guidelines set by CLIA (Clinical Laboratory Improvement Amendments) Requirements for Analytical Quality.23 Moreover, a laboratory implementing ELISA methodology for the detection of IgG and IgE food-specific antibodies must clearly identify its suitability for this purpose in yielding reproducible and consistent results for each patient tested on every occasion. Reproducibility as the name implies, is the ability of the test to reproduce the same test results for identical samples under identical test conditions. Identical testing conditions must be assured by the laboratory through day-to-day and run-to-run, for a dependable test, or a correct and precise testing procedure that has been exactly defined. Duplicate testing for example, provides an internal measure of control and assures reproducibility. If the testing method is precise there should be minimal variation between the duplicate runs. In addition to this, daily in-house blinded split sample reproducibility checks are on the onus of the lab and constitute good laboratory practice for quality assurance. Most often a laboratory also participates in periodic blinded testing through an approved accredited organization to further insure reproducibility of test results. These strict quality measures guarantee repeatability of the results; namely the presence of food-specific IgE/IgG antibodies will be consistently detected each and every time the patient’s serum sample is tested.

In order for a laboratory to provide its testing services, it must hold a license and abide by federal CLIA rules, the governing body for analytical proficiency testing criteria for acceptable analytical performance. The purpose of CLIA is to promote good laboratory practices and to assure a reliable test with reproducible and consistent results. Under the government of CLIA, a diagnostic laboratory has demonstrated and documented participation in proficiency testing and quality assurance and control. CLIA certification and accreditation requires that the laboratory be inspected by a CLIA accredited nonprofit organization, and approved by the federal Centers for Medicare and Medicaid Services (CMS), formerly HCFA (Health Care Finance Administration). Inspections for this certification may be completed through COLA (Commission of Laboratory Accreditation) or CAP (College of American Pathologists), and are often more rigorous than CLIA regulations. CLIA governs all laboratory operations including; accreditation, proficiency testing, quality assurance, quality control, records and information systems, test methods, equipment, and instrumentation. Regulations set under these operations are designed to assure reliability and consistency of laboratory test results. In the strictest sense, a laboratory must establish and follow CLIA procedures for monitoring and
validating the quality of the analytical testing process to assure reliability; a true and reputable test result. However, it is the responsibility of the laboratory in compliance with federal quality standards established by CLIA, to assure reliable laboratory results and documentation/records. More so, it is the responsibility of the health care practitioner to understand these criteria to seek a reputable testing facility.

For ELISA food allergy testing to be valid it must accurately measure what it purports to measure, namely food-specific IgG and IgE antibodies. Only in this way can it be of any clinical worth to the practitioner and patient. With respect to accuracy, accuracy expresses the closeness, or degree of agreement between a measured and established reference value. What, however, defines the established reference value as that which to compare? The presumption is that there is a true “gold standard” or an accepted method to which a new method can and should be compared to define its accuracy and hence, credibility. With respect to IgG food allergy testing, there is no “gold standard” or accepted method available to define all others. Is IgG ELISA food allergy testing therefore accurate? It can be argued that this testing method is accurate if it yields similar results to that obtained from another lab using the same sample. However, each lab abides by their own in-house validation and quality assurance measures, which may vary from lab to lab. Strictly, this implies that the results from one laboratory cannot be compared justly to that of another lab, again because there is no “gold standard” for quantification. The onus of responsibility to provide the practitioner and patient with a valid IgG food allergy test therefore lies in the hands of the laboratory to uphold good laboratory practices in compliance with specific federal quality standards established by CLIA.

Validity is the predictive significance of a test for its intended purpose. That is, the correlation between the test results and some criterion to which this test is supposed to predict. In laboratory medicine this criterion refers to a disease state or condition. In conventional circles the validity of a test is justified by its positive predictive value (PPV). That is, a remarkable or positive test result will identify a particular diseased state in a large proportion of the population, with defined signs and symptoms. A true relationship between the PPV and the prevalence of the disease/condition in the population represents the diagnostic value of the test and hence it’s worth. That is, the test when applied to the general population can efficiently identify those individuals who are likely to have the disease in question while excluding those unaffected. The diagnostic efficiency of the test is improved by utilizing it only in patients with clear clinical features suggestive of the disease. Two major factors in improving the diagnostic worth of the test are sensitive and specific achievement. Test sensitivity is defined as the percentage of people in the population with the disease state in question that have a remarkable test result. The specificity on the other hand, is defined as the percentage of people in the population without the disease who have a normal test result. Ideally, the specificity of a test with regards to the general population should be equal to 97.5% with 2.5% representing “false positives”. It is important to note however, that it is often difficult to reliably define sensitivity and specificity for a particular test, in part because of the challenge involved in defining the “reference population” on which to deduce a true generalization for the population at large. In addition, to what criteria do we define the presentation of the disease in question to justify its predictive value for substantiating test validity? This question needs to be addressed when considering the validity of IgG food allergy testing. First and foremost, as with all laboratory testing, it is a prudent assumption that a test supplements rather than substitute for clinical skills, and careful clinical assessment. No test should take the place of sound clinical decision-making. In addition, the clinician should understand the factors that influence the reliability of the test as such to guide valid decisions for patient care.

The purpose of IgG ELISA food allergy testing is to identify elevated IgG antibody levels to food antigens from a sample of the patient’s serum. An antigen is any substance that is regarded as foreign by the immune system and therefore capable of stimulating an immune response. Elevated food-specific IgG antibody levels are understood to represent IgG immune-mediated allergies to these particular food antigens. An allergy is defined as a pathological immune reaction to an antigen. With this in mind, allergy should not be defined solely as an IgE-mediated hypersensitive atopic condition: allergic rhinitis, atopic dermatitis and asthma. Allergy is any abnormal immune reaction to an allergen that may result in a broad range of
Inflammatory responses, and elevated food-specific IgG antibodies may have far-reaching systemic consequences. It is well established in immunological circles that FcγR polymorphisms play an important role in the pathogenesis of inflammatory disease. This, in association with the extended half-life of IgG antibodies make insult through dietary challenge an important issue in the management of the chronically unwell patient. Assessment of elevated IgG food-specific antibodies provide a useful tool for patient-tailored diet therapy as a means to control in part, undue FcγR-mediated effector functions in the patient with receptor polymorphisms that are implicated in disease susceptibility.

Furthermore, one cannot discuss the clinical validity of IgG food allergy testing without discussing 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). It has been argued that oral tolerance to dietary antigens is the B cell switching from IgE/IgG antibody production to IgA, under the influence of a novel cytokine profile. Abrogation of tolerance to otherwise innocuous food proteins may be involved in the pathogenesis of a variety of disease states. Loss of mucosal barrier integrity, excessive stimulation of antigen presenting cells, favor overstimulation of Th cells, and a cytokine profile that is incompatible with induction of tolerance. This loss of tolerance is the model of a variety of pathologies from autoimmune-based disease to food allergies and enteropathies; the mechanisms of which are in the forefront in clinical research today.

Mucosal Tolerance

Mucosal tolerance represents the most important response to food antigens that affords systemic hyporesponsiveness or protection from inflammatory events and bodily disorder. A tolerogenic response to dietary challenge is critical to allow for competent digestion and absorption of nutrients for maintenance of normal structure and function of the body. Loss of tolerance on the other hand, is the unfavorable immune reaction with hyperresponsiveness to daily dietary challenge. As a result, mediators for enhanced inflammation and tissue damage, both local and systemic, predominate with sequelae both acute and chronic. Moreover, hypersensitivity to ingested foods, IgG and IgE-mediated food allergies, signifies loss of oral tolerance. Celiac disease for example, is loss of tolerance to wheat gliadin, a prolamine-derived peptide fraction of the cereal protein gluten. Multiple grain allergies result, with elevated IgG antibodies to other prolamines including that of rye (secalin), and barley (hordein). This is an abnormal immune-mediated and cytotoxic reaction characterized by partial or total villous atrophy and lymphoid infiltration of the lamina propria. Crohn’s disease and ulcerative colitis also represent inflammatory bowel diseases in which there is a loss of oral tolerance, namely to commensal bacteria.

From each meal of the day the gut mucosa is bombarded with a myriad of potentially antigenic food proteins. Likewise, the diverse population of normal bacterial flora in the intestine poses an additional potential antigenic challenge. Yet, under normal and ideal circumstances, the body does not react unfavorably to these mucosal antigens. Resident microbial flora and food proteins result in immunologic silence, or tolerance. How the mucosal immune system is able to define these antigens as pathogenically important and mount an inappropriate response in any given circumstance in the susceptible individual is influenced by many factors. In the neonate for instance, improper establishment of oral immune tolerance may be influenced through genetic makeup, insufficient acquisition of microflora, early introduction of solid foods, early cessation of breast-feeding, and maternal transfer of food antigens through the breast milk. In the adult, breach of oral tolerance may be mediated through genetic makeup, insufficient acquisition of microflora, early introduction of solid foods, early cessation of breast-feeding, and maternal transfer of food antigens through the breast milk.

Moreover, any trauma or insult to the protective mucosal barrier that increases permeability may abrogate a tolerogenic response.

As a clinician a true understanding of the mucosal immune system of the gastrointestinal tract and the induction of oral tolerance, or lack thereof to dietary proteins, is key to developing a clear appreciation for the potential implications of food allergies in systemic health.

The body employs many mechanisms at the intestinal lumen-mucosa interface to prevent the induction of hypersensitivity to food proteins. The first level of protection against undue penetration of oral antigens involves non-immunological
The intestinal immune system offers a second line of defense against food antigens. 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. It is argued that IgA deficiency may predispose one to food hypersensitivity as slgA is believed to serve as a barrier to absorption, preventing the uptake of food antigens. In addition, early studies rationalize a systemic decrease in specific IgE and IgG concomitant with a local increase in slgA 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-β 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. However, the prime importance of slgA in oral tolerance is not without challenge. Experimental studies have proven it difficult to induce an IgA antibody response in animals immunized orally with protein antigens, and under normal circumstances there is negligible food specific IgA in the intestine. Shi et al, in particular, have demonstrated the suppression of OVA-specific IgA responses by fed antigen in experimentally bred mice deficient in Th1 and Th2 cells, but competent in TGF-β-mediated oral tolerance. In other words, oral tolerance in these mice did not correlate with a concomitant elevation in OVA-specific IgA. On the contrary, the IgA response was suppressed compared to that observed in normal BALB/c control mice. The reduction in IgA in other words, paralleled the reduction of systemic IgG and IgE in oral tolerance. The researchers conclude therefore a supporting role for Th1 and Th2 cytokines in regulating the induction of IgA immunity.

Contrary to these findings, Kim et al, have shown TGF-β to be co-stimulatory in IgA production, influencing B cell differentiation into IgA-producing cells. IgA is the predominant immunoglobulin secreted by the B cells of the gut. Constituting over 70% of all immunoglobulin present in the intestinal mucosa, it obviously plays a key role in immune exclusion of food antigens as a “default” mucosal B cell response. However, its position in oral tolerance is less clear. The GALT is exquisitely sensitive to the residing cytokine milieu of which dysregulation alters mucosal responsiveness. TGF-β and other immunosuppressive cytokines, including those of Th2, interact to maintain intestinal homeostasis and nonresponsiveness to innocuous food antigens. TFG-β in particular, inhibits the proliferation of T and B cells, and decreases the secretion of IgG immunoglobulins, yet at the same time, induces isotype IgA class switching. Clearly, local IgA immunity alone is unlikely to account for the absence of food hypersensitivities, but does accompany and serves as a useful backup to other more pivotal immunoregulatory mechanisms.

The Gastrointestinal Mucosa

When we consider the cellular arrangements in the gastrointestinal system it is amazing how the epithelial lining of the mucosa, connected by tight junctions, represents the primary barrier to food antigen entry. The mucosal epithelium, comprised of absorptive cells, mucus producing goblet cells, intraepithelial lymphocytes (IEL’s), and a basal membrane, is the interface between the external and internal environments of the body, and permits or excludes entry of various materials appropriately under ideal conditions.

The gastrointestinal mucosa is the largest surface of about 300m² that is in continuous contact with the external environment. Rightly so, it houses over 60% of measurable immune parameters including; mesenteric lymph nodes, Peyer’s patches (PP), isolated follicles, lamina propria lymphocytes, and IEL’s. These immune components span the epithelial lining and lamina propria and constitute the gut associated lymphoid tissues (GALT). GALT is the largest lymphoid organ of our immune system comprising 80% of the immunoglobulin producing cells in the body.
Antigen presentation in the intestinal mucosa includes; B cells, macrophages, and dendritic cells of which reside primarily in the lamina propria, PP, and mesenteric lymph nodes of the GALT. Not limited to this repertoire, antigen presentation also occurs via; mucosal T cells, IEL's, and intraepithelial cells (IEC’s). It is clear from this list that antigen sampling does not solely occur via the M cells overlying PP. All cell types are implicated in the mechanisms of oral tolerance induction. The competency in antigen presentation, the dynamics in T cell trafficking, the dose and type of antigen, and changes in the cytokine milieu of the gut, together influence the antigen-specific T helper pattern activity; either towards down-regulation of the mucosal immune response to facilitate tolerance, or towards untoward inflammation.

Other factors influencing the predominant immune response to food antigen include genetic background and indigenous gut flora. With regards to the former, celiac disease for example, is believed to be due in part to aberrant antigen presentation. Over 95% of patients with celiac disease carry a DQ2 (HLA-DQ2) gene that encodes MHC II markers that present gliadin to T cells in the lamina propria. Cytokine release increases the expression of HLA-II thus amplifying the immune response with resulting cell damage. These inflammatory mediators also increase gut permeability and promote the differentiation of B cells into IgG-antigliadin antibody-producing plasma cells.

Indigenous gut microflora has been strongly implicated in competent induction of oral tolerance. The gastrointestinal tract contains about 100,000 billion, or three and one-half pounds worth of viable microflora of which there is a variation in number and type in the different regions of the intestine. Lactobacilli predominate in the small intestine, particularly in the middle and distal ileum, whereas Bifidobacteria increase in prevalence from the cecum to large intestine. Gut microflora are compulsory to the development of mucosal immunoresponsiveness - humoral and cell-mediated immunity, during the neonatal period, and serves to prime the GALT throughout the life of the individual. Following birth, in the absence or delay of colonizing microflora, oral tolerance may be abrogated. Specifically, there is incomplete maturation and development of Peyer’s patches, intraepithelial and lamina propria lymphocytes, in addition to decreased levels of plasma cells and IgA antibody production. Clearly, defective development of the mucosal immune system in this way will incite deregulated inflammation and negatively influence the immune response to dietary antigens.

The mucosal surface represents the interface between the internal and external environments of the body that is in continual contact with a myriad of food proteins, invasive pathogens and indigenous flora on a daily basis. Discernment between infectious and noninfectious agents is therefore key to survival of the individual in his environment. Under normal circumstances down-regulation of the immune response governs oral tolerance to dietary antigens and indigenous flora of the gastrointestinal tract. The precise mechanisms involved in inducing oral tolerance to dietary antigens are imperfectly known. It is important to keep in mind that oral tolerance is a complex immune response that involves a precarious balance among several immune-mediated parameters. A glimpse into the competency of tolerance through IgG food allergy testing via the ELISA method is a simple tool for the practitioner to visualize the immunological response to dietary challenge in the patient. In practice, this assessment may guide treatment to nullify undue mediators of inflammation in the body that may be perpetuating a disease process.
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