

## **GN1 Toward an understanding of Allergy and In-Vitro Testing**

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Food represents the largest antigenic challenge facing the immune system. Assuming complete digestion, an intact intestine, a sturdy constitution, and minimal antigenic exposure such that the immune system is not overwhelmed, all goes well. Weaknesses in one or more of these areas, however, can result in immune attacks upon foods as if they were foreign invaders. A long list of conditions have been associated with food reactions including fatigue, migraine, irritable bowel disease, gallbladder disease, arthritis, asthma, rhinitis, ADHD, enuresis, epilepsy, eczema, psoriasis, aphthous ulcers and recurrent sinusitis, otitis media and other infections.

## **GN2 Reported food Intolerance and respiratory symptoms**

R.K.Woods, M.Abramson, J.M.Raven, M Bailey, JM Weiner, E.H.Walters. Eur Respir J 1998 11:151-155

The aim of the study was to assess the ability of the European Community Respiratory Health Survey (ECRHS) questionnaire to provide data on the prevalence, type and reported symptoms associated with food intolerance from a group of young adults in Melbourne. Six hundred and sixty nine randomly selected subjects completed the questionnaire with 553 attending the laboratory for skin-prick tests, anthropometry, and ventilatory function tests. A further 207 symptomatic participants completed the questionnaire, with 204 of them attending the laboratory. Seventeen per cent of all respondents reported food intolerance or food allergy. A wide variety of food items was cited as being responsible for food-related illnesses. Those with current asthma did not report food-related illness more frequently than those without asthma. Respondents who reported respiratory symptoms following food ingestion were more likely to be atopic, to have used inhaled respiratory medications in the previous 12 months, reported less exposure to regular passive smoking over the past 12 months and weighed more. These associations between respiratory symptoms and food intolerance require further prospective investigation and verification. The importance of using appropriate dietary methodology in future studies for determining diet-disease relationships was highlighted by this study.

## **GN4 The clinical relevance of IgG food allergy testing through ELISA (Enzyme-Linked Immunosorbent Assay)**

Townsend Letter for Doctors and Patients | Date: 1/1/2004 | Author: Suen, Raymond M.; Gordon, Shalima

Allergic reactions to foods may be classified as either IgE-mediated or nonIgE-mediated--the role of the former in food allergy being well-established. However, interestingly enough, the majority of food allergies are associated with specific nonIgE-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 look for allergen-specific IgE antibodies from the patient's serum. This poses considerable limitations in the clinical assessment of the chronically unwell patient.

## **GN5 IgG mediated food allergy as trigger of fibromyalgia complaints and the influence of an elimination diet**

Mario Krause, Rotenburg a. d. Fulda 2005. Faculty of Ludwig Maximilian University of Munich

Functional and psychovegetative complaints of fibromyalgia patients (e.g. polytopic pain, stress intolerance, lassitude, migraines, depressions, sleep disorders, rapid fatigability, etc.) and especially irritable colon are similar to the complaints of patients with food allergies or food intolerances. Specific histological changes are absent up to now; cellular inflammatory signs cannot be detected. A deterioration of symptoms is frequently found with intermittent inflammatory illnesses. Spontaneous remissions are described, but this frequently concerns a chronic clinical picture. Physiotherapy procedures, heat applications, antidepressants, NSAID or corticoids will normally be employed for therapy.

## **GN6 Gastrointestinal Candida colonisation promotes sensitisation against food antigens by affecting the mucosal barrier in mice**

N Yamaguchi, R Sugita, A Miki, N Takemura, J Kawabata, J Watanabe, K Sonoyama. Gut 2006;55:954-960. Doi: 10.1136/gut.2005.084954

Backgrounds and aims: Controversy still exists as to whether gastrointestinal colonisation by *Candida albicans* contributes to aggravation of atopic dermatitis. We hypothesised that *Candida* colonisation promotes food allergy, which is known to contribute to a pathogenic response in atopic dermatitis. We tested this using a recently established murine *Candida* colonisation model. Methods: *Candida* colonisation in the gastrointestinal tract was established by intragastric inoculation with *C. albicans* in mice fed a synthetic diet. To investigate sensitisation against food antigen, mice were intragastrically administered with ovalbumin every other day for nine weeks, and antiovalbumin antibody titres were measured weekly. To examine gastrointestinal permeation of food antigen, plasma concentrations of ovalbumin were measured following intragastric administration of ovalbumin. Results: Ovalbumin specific IgG and IgE titres were higher in BALB/c mice with *Candida* colonisation than in normal mice. Gastrointestinal permeation of ovalbumin was enhanced by colonisation in BALB/c mice. Histological examination showed that colonisation promoted infiltration and degranulation of mast cells. *Candida* colonisation did not enhance ovalbumin permeation in mast cell deficient W/W<sup>v</sup> mice but did in congenic littermate control +/+ mice. Reconstitution of mast cells in W/W<sup>v</sup> mice by transplantation of bone marrow derived mast cells restored the ability to increase ovalbumin permeation in response to *Candida* colonisation. Conclusions: These results suggest that gastrointestinal *Candida* colonisation promotes sensitisation against food antigens, at least partly due to mast cell mediated hyperpermeability in the gastrointestinal mucosa of mice.

## **GN7 Ovalbumin-specific immunoglobulin G and subclass responses through the first 5 years of life in relation to duration of egg sensitization and the development of asthma**

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**Background** Egg sensitization, particularly persistent sensitization, is a risk factor for later asthma. However, little is known about accompanying IgG and subclass responses and how they might relate to asthmatic outcome. **Objective** To characterize hen's egg ovalbumin (OVA) IgG and subclass responses through the first 5 years of life in relation to duration of egg sensitization and later asthma. **Subjects and methods** The subjects (n=46) formed part of a larger cohort, born to atopic parents, who had been evaluated prospectively for the development of asthma. Egg sensitization was classified as transient (positive egg skin prick test at 1 year only) or persistent (positive skin test for at least 2 years). Plasma OVA IgG, IgG1 and IgG4 concentrations at birth (cord), 6 months, 1 and 5 years of age were measured by ELISA. **Results** The kinetics of OVA IgG and IgG1 responses, but not IgG4, differed between egg sensitized and non-egg sensitized (NES) children. Only persistently sensitized children had a rise in OVA IgG1 concentration through the first year of life, and at 1 year of age they had significantly higher OVA IgG and IgG1 than either transiently sensitized or NES children. High OVA IgG1 was associated with later asthma: at 1 year of age, OVA IgG1 greater than 14 500 U predicted asthma with a sensitivity 64% and specificity 74%. **Conclusion** OVA IgG and subclass responses relate to the duration of egg sensitization. Measurement of OVA IgG1 concentration in infancy might offer a useful adjunct to identify those at an increased risk of asthma.

## **GN8 Inflammatory mediators in the elderly.**

Krabbe KS, Pedersen M, Bruunsgaard H. Department of Infectious Diseases, H:S, Rigshospitalet, University of Copenhagen, M7641, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.

Exp Gerontol. 2004 May;39(5):687-99.

Ageing is accompanied by 2-4-fold increases in plasma/serum levels of inflammatory mediators such as cytokines and acute phase proteins. A wide range of factors seems to contribute to this low-grade inflammation, including an increased amount of fat tissue, decreased production of sex steroids, smoking, subclinical infections (e.g. asymptomatic bacteriuria), and chronic disorders such as cardiovascular diseases and Alzheimer's disease. Furthermore, there is some evidence that ageing is associated with a dysregulated cytokine response following stimulation. Several inflammatory mediators such as tumour necrosis factor- $\alpha$  and interleukin-6 have the potential to induce/aggravate risk factors in age-associated pathology, providing a positive feedback mechanism. Thus, it is possible that inflammatory mediators constitute a link between life style factors, infections and physiological changes in the process of ageing on the one hand and risk factors for age-associated diseases on the other. Consistent with this, inflammatory mediators are strong predictors of mortality independently of other known risk factors and co-morbidity in elderly cohorts. A direct pathogenetic role of inflammatory mediators would be highly likely if longevity was shown to be associated with cytokine polymorphisms regulating cytokine production. Several studies support indeed this hypothesis but, unfortunately, findings in this area are conflicting, which probably reflects the complexity of the effect of cytokine polymorphisms and their interaction with the lifestyle and sex.

## **GN9 The Role of Hidden Food Allergy/Intolerance in Chronic Disease**

Alan R. Gaby, M.D. *Alt Med Rev* 1998;3(2):90-100

A large body of medical literature has indicated that hidden food allergy is a frequent cause of a wide range of physical and mental conditions. Hidden allergies can be “unmasked” by means of an elimination diet, followed by individual food challenges. Although the concept of hidden food allergy remains controversial, the evidence strongly suggests that identification and avoidance of allergenic foods can relieve a number of common and difficult-to-treat medical problems. (*Alt Med Rev* 1998;3(2):90-100).

## **GN10 Patterns of immunoglobulin G responses to egg and peanut allergens are distinct: ovalbumin-specific immunoglobulin responses are ubiquitous, but peanut-specific immunoglobulin responses are up-regulated in peanut allergy.**

Tay SS, Clark AT, Deighton J, King Y, Ewan PW. Department of Medicine, Cambridge University, Cambridge, UK. *Clin Exp Allergy* 2007 Oct;37(10):1512-8.

**Background** The clinical significance of food-specific IgG subclasses in food allergy and tolerance remains unclear. Specific IgG titres are often reported in non-standardized units, which do not allow comparisons between studies or allergens. **Objective** To quantify, in absolute units, ovalbumin (OVA)- and peanut-specific IgG levels in children with peanut or egg allergy (active or resolved) and in non-allergic controls. **Methods** Children aged 1–15 years were recruited. Peanut allergy was diagnosed by convincing history and a 95% predictive level of specific IgE; egg allergy or resolution was confirmed by oral challenge. Serum IgG, IgG1 and IgG4 levels (mg/mL) to OVA and peanut extract were quantified by ELISA. **Results** OVA- and peanut-specific IgG was detected in all subjects. In non-allergic controls (n = 18), OVA-specific IgG levels were significantly higher than peanut-specific IgG (median mg/mL IgG = 15.9 vs. 2.2, IgG1 = 1.3 vs. 0.6, IgG4 = 7.9 vs. 0.7; Po0.01). There were no differences in OVA-specific IgG, IgG1 and IgG4 between egg-allergic (n = 40), egg-resolved (n = 22) and control (n = 18) subjects. In contrast, peanut-specific IgG (median mg/mL IgG = 17.0, IgG1 = 3.3, IgG4 = 5.2) were significantly higher in peanut-allergic subjects (n = 59) compared with controls and with non-peanut-sensitized but egg-allergic subjects (n = 26). Overall, the range of IgG4 was greater than IgG1, and IgG4 was the dominant subclass in 460% of all subjects. **Conclusion** OVA-specific IgG levels of egg-allergic, egg-resolved or control groups are not distinguishable. Higher peanut-specific IgG levels are associated with clinical allergy, but the range of IgG titres of the allergic and control groups overlapped. Hence, OVA and peanut-specific IgG measurements do not appear to be of diagnostic value. Strong IgG responses to OVA may be a normal physiological response to a protein frequently ingested from infancy, whereas up-regulated IgG responses in peanut allergy may be indicative of a dysregulated immune response to peanut allergens.

## **GN11 Serum IgG responses to food antigens in the Italian population evaluated by highly sensitive and specific ELISA test**

Volpi N, Maccari F. *J Immunoassay Immunichem* 2009;30(1):51-69. Department of Biologia Animale, Biological Chemistry Section, University of Modena and Reggio Emilia, Modena, Italy

Using an optimized and validated ELISA method, we performed a serum test for assaying the binding capacity of serum IgG to proteins extracted from approx. 160 different foods to investigate the reactivity of specific IgG antibodies in the Italian population composed of 6,879 subjects (4,551 females and 2,328 males). 44 antigens showed an IgG response greater than 10% and only 14 aliments had an elevated reactivity greater than 20%, in particular, milk, from cow and goat, and several milk derivatives, along with egg albumen and yeasts. The IgG response to the high reactive food antigens depending on the age of the 6880 subjects was also analyzed. We demonstrated a high IgG response in a very large subject group to milk and milk derivatives, and egg albumin antigens, and we conclude that the validated ELISA test may be applied for the serum/plasma IgG antibody level determination as a useful indicator of adverse reactions to food and food hypersensitivity.

## **GN12 Detection of IgE, IgG, IgA and IgM antibodies against raw and processed food antigens**

Vojdani A. *Nutra Metab* London 2009 May 12;6:22 822 S, Robertson Blvd, Ste, 812, Los Angeles, CA 90035, USA

Background: Despite the first documented case of food allergy to cooked food in 1921 by Prausnitz and Kustner, all commercial food antigens are prepared from raw food. Furthermore, all IgE and IgG antibodies against dietary proteins offered by many clinical laboratories are measured against raw food antigens. Methods: We developed an enzyme-linked immunosorbent assay for the measurement of IgE, IgG, IgA and IgM antibodies against raw and processed food antigens. Sera with low or high reactivity to modified food antigens were subjected to myelin basic protein, oxidized low density lipoprotein, and advanced glycation end products (AGE) such as AGE-human serum albumin and AGE-hemoglobin. Results: Compared to raw food antigens, IgE antibodies showed a 3–8-fold increase against processed food antigens in 31% of the patients. Similarly, IgG, IgA and IgM antibodies against modified food antigens overall were found at much higher levels than antibody reactions against raw food antigens. Almost every tested serum with high levels of antibodies against modified food antigens showed very high levels of antibodies against myelin basic protein, oxidized low density lipoprotein, AGE-human serum albumin and AGE-hemoglobin. Conclusion: We conclude that the determination of food allergy, intolerance and sensitivity would be improved by testing IgE, IgG, IgA and IgM antibodies against both raw and processed food antigens. Antibodies against modified food antigens, by reacting with AGEs and tissue proteins, may cause perturbation in degenerative and autoimmune diseases such as diabetes, atherosclerosis, inflammation, autoimmunity, neurodegeneration and neuroautoimmunity.

## **GN13 Testing for food reactions: the good, the bad, and the ugly**

Mullin GE, Swift KM, Lipski L, Turnbull LK, Rampertab SD. *Nutr Clin Pract* 2010 Apr;25(2):192-8. Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

An increasing number of commercial tests for food allergies are marketed to consumers and healthcare practitioners with tenuous claims. The aim of this article is to provide an evidence-based review of the tests and procedures that currently are used for patients with suspected food allergy. A systematic review of the literature evaluating the validity of tests and procedures used in food reactions was performed using conventional search engines (eg, PubMed, Ovid) as well as consumer sites (eg, Google, Bing). The National Library of Medicine Medical Subject Headings (MeSH) term food hypersensitivity was used along with food allergy testing, food sensitivity testing, food intolerance testing, and adverse food reactions. Of the results obtained, testing for immunoglobulin E (IgE)-mediated food allergy was best represented in PubMed. IgE-based testing continues to be the gold standard for suspected food allergies. Among modalities used by many conventional and alternative practitioners, immunoglobulin G (IgG)-based testing showed promise, with clinically meaningful results. It has been proven useful as a guide for elimination diets, with clinical impact for a variety of diseases. Mediator release testing and antigen leukocyte cellular antibody testing were only represented on consumer sites. Further investigation into the validity and the clinical application of these tests and procedures is required. Disclosing the basis for food reactions continues to present a diagnostic challenge, and testing for food allergies in the context of an appropriate clinical history is paramount to making the correct diagnosis.

## **GN14 Serum antibodies to dietary antigens in patients with HIV-1 infection**

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Diarrhoea is a common complication of HIV-1 infection [1], but in a considerable proportion of patients, especially in earlier stages of the disease, the cause of diarrhoea remains unclear despite extensive investigation [2]. Increased levels of serum antibodies against food proteins have been described in HIV-infected children [3], similar to patients with non-IgE-mediated gastrointestinal food hypersensitivity [4], coeliac disease [5], or chronic inflammatory bowel disease [6]. These abnormalities are thought to result from increased gut permeability leading to increased uptake of dietary antigens and an aberrant mucosal IgG response in these patients [7]. Increased intestinal permeability [8] and an increase of IgG in mucosal secretions [9] have also been found in patients infected with HIV-1. Since abnormal immunity to dietary antigens may contribute to the pathogenesis of diarrhoea, we investigated serum antibodies to food proteins in HIV-1-infected patients with and without diarrhoea.

## **GN15 Increase in intranuclear nuclear factor KB and decrease in inhibitor KB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect1–3**

Ahmad Aljada, Priya Mohanty, Husam Ghanim, Toufic Abdo, Devjit Tripathy, Ajay Chaudhuri, Paresh Dandona. *Am J Clin Nutr* 2004;79:682–90.

Background: In view of the stimulatory effect of glucose on reactive oxygen species (ROS) generation, we investigated the possibility that a mixed meal stimulates ROS generation and possibly induces concomitant proinflammatory changes. Objective: The objective was to determine whether the intake of a 900-kcal mixed meal induces an increase in ROS generation by leukocytes and an inflammatory response at the cellular level. Design: Nine normal-weight subjects were given a 900-kcal mixed meal, and 8 normal-weight subjects were given 300 mL water after an overnight fast. Blood samples were collected at 0, 1, 2, and 3 h. ROS generation by mononuclear cells and polymorphonuclear leukocytes and the expression of p47phox subunit were measured. Intranuclear nuclear factor B (NF-B) binding and the expression of inhibitor B[1] (IB[1]), IB kinase [1] (IKK[1]), and IB kinase (IKK) were measured. Plasma concentrations of C-reactive protein (CRP) and soluble intercellular adhesion molecule were also measured. Results: ROS generation by mononuclear cells and polymorphonuclear leukocytes and p47phox expression increased significantly. The expression of IKK[1] and IKK and DNA-binding activity of NF-B increased significantly, whereas IB[1] expression decreased. Plasma CRP concentrations increased. The intake of 300 mL water did not induce a change in any of the above indexes. Conclusions: These data show that the intake of a mixed meal results in significant inflammatory changes characterized by a decrease in IB[1] and an increase in NF-B binding, plasma CRP, and the expression of IKK[1], IKK, and p47phox subunit. These proinflammatory changes are probably relevant to the state of chronic hypertension and obesity and to its association with atherosclerosis.

## **GN16 Inflammatory mediators in the elderly.**

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Ageing is accompanied by 2-4-fold increases in plasma/serum levels of inflammatory mediators such as cytokines and acute phase proteins. A wide range of factors seems to contribute to this low-grade inflammation, including an increased amount of fat tissue, decreased production of sex steroids, smoking, subclinical infections (e.g. asymptomatic bacteriuria), and chronic disorders such as cardiovascular diseases and Alzheimer's disease. Furthermore, there is some evidence that ageing is associated with a dysregulated cytokine response following stimulation. Several inflammatory mediators such as tumour necrosis factor- $\alpha$  and interleukin-6 have the potential to induce/aggravate risk factors in age-associated pathology, providing a positive feedback mechanism. Thus, it is possible that inflammatory mediators constitute a link between life style factors, infections and physiological changes in the process of ageing on the one hand and risk factors for age-associated diseases on the other. Consistent with this, inflammatory mediators are strong predictors of mortality independently of other known risk factors and co-morbidity in elderly cohorts. A direct pathogenetic role of inflammatory mediators would be highly likely if longevity was shown to be associated with cytokine polymorphisms regulating cytokine production. Several studies support indeed this hypothesis but, unfortunately, findings in this area are conflicting, which probably reflects the complexity of the effect of cytokine polymorphisms and their interaction with the lifestyle and sex.