Food Allergy In Adults

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INEKO AB

THIS THESIS IS DEDICATED TO ALL PATIENTS WITH
ADVERSE REACTIONS TO FOODS

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Institute of Medicine at Sahlgrenska Academy in the University of Gothenburg, Sweden, 2015

ABSTRACT

The knowledge on adverse reactions to foods and the spectrum of food-related gastrointestinal symptoms in relation to allergy in adults is still scarce. The conventional allergy tests do not always offer with precision an accurate diagnosis in case of suspicious food allergy and therefore patients often need to be investigated further with oral food challenge or even intestinal biopsy.

Adult patients with pollen allergy with and without gastrointestinal symptoms were investigated with intestinal biopsies during and outside the birch pollen season for exploring the pattern of mucosal allergic inflammation.
Patients with severe allergy and subjects sensitized to peanuts were investigated with the basophil activation tests in terms of assessing the use of this new diagnostic tool in case of food allergy in adults.
The prevalence of adverse reactions to different foods and the prevalence for food-related gastrointestinal symptoms along with the IgE-sensitization profile for the most common foods were determined among adults with asthma as part of the larger West Sweden Asthma Study.

Interestingly, the results show that adults allergic to birch pollen, present prominent intestinal allergic inflammation during the birch pollen season, and for the first time, clear signs of ongoing season-related intestinal allergic inflammation is revealed, in adults without any gastrointestinal symptomatology.
The basophil activation test may be used as complementary diagnostic tool in case of severe peanut allergy, and for discriminating these patients from peanut sensitized subjects.
The novelty of the last study was that the prevalence for self-reported adverse reactions, and gastrointestinal symptoms to foods, is much higher among asthmatics compared to non-asthmatics. Furthermore asthmatics were significantly more often sensitized to birch related food items, and hazelnut was the food that adults with asthma most commonly experienced adverse reactions to.

Keywords: intestinal allergy, birch pollen, basophil activation test, peanut allergy, prevalence, asthma

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SAMMANFATTNING PÅ SVENSKA

Kunskapen om prevalensen för de ogynsamma födoämnesreaktioner och födoämnesrelaterade gastrointestinala besvär hos vuxna allergiker är fortfarande vetenskapligt bristfälligt. De konventionella allergitester tillför inte alltid med precision en korrekt diagnostik i fall av misstänkt födoämnesallergi och därför patienterna ofta behöver att genomgå kompletterande utredning med en födoämnesprovokation eller även intestinal biopsi.

Vuxna pollenallergiska patienter såväl med som utan gastrointestinala besvär genomgick utredning med intestinala biopsier under respektive utanför björkpollen säsongen för att utforska mönstret av allergiska inflammationen i intestinala slemhinnan.
Patienter med allvarlig jodnötsallegi och vuxna sensibiliserade mot jordnötter, utreddes med basofilaktiveringstestet för att undersöka om det testet kan användas i diagnostiken.
Prevalensen för de ogynsamma reaktioner mot olika födoämnen, för de födoämnesrelaterade gastrointestinala symtom samt IgE-sensibiliseringsprofilen för de vanligaste födoämnen hos vuxna astmatiker, undersöktes som en del av den omfattade West Sweden Astma Studien.

Vuxna björkpollen allergiska patienter uppvisar en ökad allergisk inflammation i intestinala slemhinnan under björkpollen säsongen, och för första gången, tydliga tecken av pågående säsongsbunden intestinal allergisk inflammation avslöjas även hos vuxna utan någon gastrointestinal symtomatologi över huvudtaget.
Basofilaktiveringstestet kan användas som kompletterande diagnostiskt verktyg i fall av allvarlig jordnötsallergi och kan urskilja jordnötsallergiska från jordnötssensibiliserade vuxna.
Den sista studien visar att prevalensen av födoämnesintolerans är signifikant högre hos astmatiker jämfört med icke-astmatiker. Vuxna astmatiker rapporterar oftare ogynnsamma reaktioner och gastrointestinala symtom från björkrelaterade födoämnen där hasselnöt var det vanligaste födoämnet för vilket det uppvisades IgE-sensibilisering i mycket högre frekvens jämfört med icke-astmatiker.

ΠΕΡΙΛΗΨΗ ΣΤΑ ΕΛΛΗΝΙΚΑ

Η γνώση και οι μελέτες για την επιδημιολογία της τροφικής δυσανεξίας καθώς και των γαστρεντερικών διαταραχών από τα διάφορα τρόφιμα, είναι ακόμη επιστημονικά ελλιπείς στους ενήλικες ασθενείς με άσθμα. Τα συμβατικά τεστ που χρησιμοποιούνται κατά την αλλεργιολογική διερεύνηση, συχνά δεν είναι επαρκή, ώστε να προσφέρουν την σωστή διάγνωση με ακρίβεια. Για το λόγο αυτό, είναι συχνά απαραίτητο οι ασθενείς να υποβάλλονται στην διαδικασία της τροφικής πρόκλησης ως συμπληρωματική διαγνωστική μέθοδο ή ακόμη και σε γαστροσκόπηση με βιοψίες του εντερικού βλεννογόνου.

Ενήλικες ασθενείς με και χωρίς γαστρεντερικά συμπτώματα, αλλά και αλλεργία στην γύρη της σημύδας κατά την περίοδο της άνοιξης, διερευνήθηκαν με γαστροσκοπήσεις κατά την διάρκεια και εκτός της περιόδου της σημύδας, με σκοπό να εξεταστεί ο τύπος της γαστρεντερικής αλλεργικής φλεγμονής σε βιοψίες του εντερικού βλεννογόνου.
Ασθενείς με σοβαρή αλλεργία καθώς και ενήλικες ευαισθητοποιημένοι στο φιστίκι διερευνήθηκαν με ένα νέο διαγνωστικό τεστ, το τεστ διέγερσης βασεόφιλων, με σκοπό να εκτιμηθεί η χρήση του στη διαγνωστική της τροφικής αλλεργίας.
Η εκτίμηση των επιδημιολογικών στοιχείων όσον αφορά την επικράτηση της τροφικής δυσανεξίας, τις αντιδράσεις και τις γαστρεντερικές διαταραχές οφειλόμενα σε διάφορα τρόφιμα καθώς και η καταγραφή του προφίλ των αντισωμάτων IgE στα πιο συνηθισμένα τρόφιμα σε ενήλικες με άσθμα διερευνήθηκε σε μέρος του πληθυσμού στην εκτεταμένη μελέτη West Sweden Asthma Study.

Ενδιαφέροντα ήταν τα αποτελέσματα των βιοψιών από τον εντερικό βλεννογόνο, τα οποία έδειξαν ότι ενήλικες με αλλεργία στην γύρη κατά την περίοδο της σημύδας την άνοιξη, παρουσιάζουν στοιχεία αυξημένης αλλεργικής φλεγμονής στον εντερικό βλεννογόνο. Επίσης, για πρώτη φορά παρατηρείται το γεγονός ότι ακόμη και αλλεργικοί χωρίς γαστρεντερικές διαταραχές παρουσιάζουν ξεκάθαρα σημάδια εν εξελίξει αλλεργικής γαστρεντερικής φλεγμονής κατά την περίοδο την σημύδας την άνοιξη.
Το τεστ διέγερσης βασεόφιλων μπορεί να χρησιμοποιηθεί ως συμπληρωματική διαγνωστική μέθοδος στην περίπτωση της σοβαρής αλλεργίας στο φιστίκι και μπορεί να διαχωρίσει ασθενείς με σοβαρή αλλεργία από ευαισθητοποιημένους ενήλικες στο φιστίκι.
Τα νέα ενδιαφέροντα αποτελέσματα της τρίτης μελέτης, δείχνουν ότι οι αναφορές αντιδράσεων σε διάφορα τρόφιμα, είναι πιο συχνές στους ενήλικες με άσθμα από τους μη ασθματικούς. Οι ασθματικοί αναφέρουν συχνότερα αντιδράσεις στο φουντούκι καθώς επίσης και στα τρόφιμα που παρουσιάζουν διασταυρούμενη αντίδραση με την γύρη κατά την περίοδο της σημύδας, στα οποία επίσης είναι ευαισθητοποιημένοι στα IgE τεστ. Αυξημένες γαστρεντερικές διαταραχές συναντώνται στους ασθματικούς συγκριτικά με τους μη ασθματικούς.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Rentzos G**, Lundberg V, Stotzer P-O, Pullerits T, Telemo E.

Intestinal allergic inflammation in birch pollen allergic patients in relation to pollen season, IgE sensitization profile and gastrointestinal symptoms.

Clinical and Translational Allergy 2014, 4:19

II. **Rentzos G**, Lundberg V, Lundqvist C, Rodrigues R, van Odijk J, Pullerits T. Telemo E.

Diagnosis of peanut allergy with basophil activation test in adults.
Submitted for publication

III. **Rentzos G,** Johanson L, Sjölander S, Telemo E, Ekerljung L.

Self-reported adverse reactions and IgE sensitization to common foods in adults with asthma.
Submitted for publication

Related publications not included in this Thesis:

Jansson SA, Heibert-Arnlind M, Middelveld RJ, Bengtsson UJ, Sundqvist AC, Kallström-Bengtsson I, Marklund B, **Rentzos G**, Akerström J, Ostblom E, Dahlén SE, Ahlstedt S.
Health-related quality of life, assessed with a disease-specific questionnaire, in Swedish adults suffering from well-diagnosed food allergy to staple foods.
Clin Transl Allergy. 2013 Jul 1;3:21.

Jansson SA, Protudjer JL, Arnlind Heibert M, Bengtsson U, Kallström-Bengtsson I, Marklund B, Middelveld RJ, **Rentzos G**, Sundqvist AC, Akerström J, Ostblom E, Dahlén SE, Ahlstedt S.
Socioeconomic evaluation of well-characterized allergy to staple foods in adults.
Allergy. 2014 Sep;69(9):1241-7.

Protudjer JL, Jansson SA, Heibert Arnlind M, Bengtsson U, Kallström-Bengtsson I, Marklund B, Middelveld R, **Rentzos G**, Sundqvist AC, Åkerström J, Östblom E, Dahlén SE, Ahlstedt S.
Household costs associated with objectively diagnosed allergy to staple foods in children and adolescents.
J Allergy Clin Immunol Pract. 2015 Jan-Feb;3(1):68-75.

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ABBREVIATIONS

APC	Antigen presenting cells
BALT	Bronchial associated lymphoid tissue
BAT AC50	Basophil allergen threshold sensitivity, as the lowest allergen concentration that was able to activate 50% of the basophils that were activated in the stimulation control
BAT	Basophil activation test
CCL-	C chemokine ligand
CCR	C chemokine receptor
CD	Coeliac disease
CRD	Component resolve diagnostics
DAO	Diamine oxidase
DBPCFC	Double blind placebo controlled food challenge
DCs	Dendritic cells
EoE	Eosinophilic esophagitis
FDEIA	Food-exercise-induced allergic reaction/anaphylaxis
fMPL	N-formyl-Met-Leu-Phe
FoxP3	Forkhead box P3
FPIES	Food protein induced enterocolitis
GALT	Gastrointestinal associated lymphoid tissue
GI	Gastrointestinal
IBD	Inflammatory bowel disease
IBS	Irritable bowel disease
IECs	Intestinal epithelial cells
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IL-	Interleukin-
ILFs	Isolated Lymphoid follicles
ISU	ISAC standardized units
LP	Lamina propria
LPLs	Lamina propria lymphocytes
LTC	Cysteinyl leukotriene
LTP	Lipid transfer protein
mAbs	Monoclonal antibodies
MAdCAM	Mucosal vascular addressin cell adhesion molecule 1
MALT	Mucoid associated lymphoid tissue
MCH	Major histocompatibility complex

MLN	Mesenteric lymph nodes
OAS	Oral allergy syndrome
OFC	Open food challenge
PGD	Prostaglandins
PP	Peyer's patches
sIgE	Specific Immunoglobulin E
SPT	Skin prick test
TCR	T-cell receptor
TGF-β	Transforming growth factor-β
TNF	Tumor necrosis factor
Tregs	T-regulatory cells

DEFINITIONS IN SHORT

Allergic response:	An aberrant, misguided immune response to an otherwise harmless antigen
Food Allergy:	An immune system reaction that occurs soon after ingesting a certain food. Even a tiny amount of the allergy-causing food can trigger signs and symptoms such as gastrointestinal symptoms hives or swollen airways or even a life-threatening reaction in some people. The reaction is caused by an immunological reaction which may be IgE- or non-IgE-mediated
Food Intolerance/ Pseudo-allergy:	An adverse reaction to food which is caused by a non-immunological mechanisms. The allergy tests are always negative. Pseudo-allergies may be caused by a metabolic effect (enzymatic deficiency, histamine reaction), toxic effect (infection) or psychogenic response
Anaphylaxis:	A severe, life-threatening, generalized or systemic hypersensitivity reaction. Acute onset of the reaction affecting two or more organ systems (skin mucosa/ respiratory tract/ cardiovascular system/ gastrointestinal tract) and/or hypotension after exposure to known allergen

Asthma:	A chronic inflammatory disorder of the airways with hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment.
Rhinoconjuctivitis:	Symptoms caused by immunologically mediated (often IgE-dependent) inflammation after exposure of the conjunctival and nasal mucous membranes to the offending allergens. Symptoms include conjunctival itching and swelling, rhinorrhea, nasal obstruction or blockage, nasal itching, sneezing and postnasal drip that reverse spontaneously or after treatment.
Eczema:	A chronic, inflammatory skin condition that is commonly found in children, but continues to present a significant health burden in adult life. Eczema is characterized by dry and itchy skin with involvement of skin creases and is often associated to a personal history of allergic disease
Basophil Activation Test (BAT):	A test used for the diagnosis of IgE mediated allergy by measuring activation markers on the surface of basophils, after in vitro stimulation with the suspicious allergen

1 INTRODUCTION

Food allergy is one of the leading and most complicated pathological conditions in the field of allergic diseases. Allergy to foods is reported already in the ancient times. At about 400 BC Hippocrates reported about the negative effects that food could have on different people and specifically about cheese allergy. Over the years, many cases of adverse reactions to foods were reported and many of them were attributed to food intoxications. It was already addressed by Dr. Matthew Baillie 200 years ago that physicians were able to treat their patients' illnesses by specific diet manipulation (1). In the early 1900's many medical writings supported the fact that foods are a problem for some individuals and can cause a whole host of medical illnesses and diseases (2). In 1905 Dr. Hare published a book titled "The Food Factor in Disease" which was a result of his observations that migraine headache was relieved when a patient followed a special diet that largely excluded fats, carbohydrates and saccharine alcoholic drinks (3). Dr. Hare sought to explain that a whole host of diseases were related to food allergies including migraine, asthma, gout, nervousness, epilepsy, mania, dyspepsia, headache, bronchitis, eczema, hypertension, gastrointestinal disturbances and other degenerative diseases (2). Dr. Alfred Schofield wrote in 1908 about successfully treating a boy who suffered from angioedema and asthma due to allergy by gradual increase of pills containing raw egg and grains of calcium lactate during an 8 month successful treatment period (4). In 1912 the pediatrician Oscar Mendersson Schloss was the first to diagnose allergy to hen's egg white by skin tests (scratch test) (5). In 1917 an article was published in the Journal of Urology, that described six patients who reacted to foods with symptoms of urticaria and renal insufficiency (2). In 1956 Dr. Coca observed that exposure to food allergens could cause changes in the pulse of the human body. This observation was published as "The Pulse Test" which outlined a direct relation between food allergies and backaches, headaches, epilepsy, diabetes, ulcers, hemorrhoids, migraine, obesity, hives, high blood pressure, depression and even multiple sclerosis with the most interesting case histories and references to successfully treated patients (6). In 1951, a book titled "Food Allergy" was published, which was covering the nature and concept of food allergy, and rotary diet (7). Finally, valuable contributions with articles, books and lectures in meetings, in the field food and digestive allergy, came at the same period from Dr. Theron Randolph (2). During the 1960's the discovery of IgE by Ishizaka and SGO Johansson represented a major advancement in allergy diagnostics and especially in the field of food allergy (8, 9).

1.1 Classification of food allergy and adverse reactions

Food allergy is defined as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food", according to the National Institutes of Allergy and Infectious Diseases (NIAID)-sponsored guidelines, meaning that, all adverse reactions from foods are not food allergies (10). Generally adverse reactions to foods are classified as toxic and non-toxic. Non-toxic reactions can be of immunologic or non-immunologic origin. Immunological reactions can be either allergic or non-allergic while allergic reactions may be either IgE-mediated or non-IgE-mediated. The IgE-mediated allergies may be primary or so called "true allergy" or secondary due to a cross-reaction with related antigens in food items, e.g. inhalation allergens (i.e. birch-hazelnut-fruit-vegetable syndrome, mugwort-celery-spice syndrome or latex-fruit syndrome) (11). IgE-mediated allergies include the food-dependent-exercise-induced allergy- or anaphylaxis (FDEIA). Celiac disease (CD) is an immunologic, but non-IgE-mediated disease, with a T cell driven immune reaction to food items containing gluten, mainly from wheat (12). There are also food allergies which are not IgE-mediated, e.g. food protein-induced enterocolitis syndrome (FPIES) with typical symptoms of vomiting, diarrhea and hypotension usually within two hours after ingestion of the offending food. This condition is mainly seen in children, but lately, case-reports, show that FPIES may rarely affect even adults (13, 14). In addition, there are conditions that may be of both mixed IgE-mediated and non-IgE-mediated mechanisms as eosinophilic esophagitis, eosinophilic gastroenteritis or eosinophilic proctitis (15, 16). In non-immunologic reactions are included adverse reaction from foods with high content of vasoactive/biogenic amines e.g. histamine, tyramine, serotonine etc. or foods with histamine-release effect (17) as well as adverse reactions from different food additives e.g. benzoic acid, colorants, stabilizers, thickeners or emulsifiers etc. (18). Also, lactose intolerance is a deficiency of lactase, which leads to gastrointestinal symptoms after ingestion of lactose (19). Finally, food items may cause health effects in other ways such as food poisoning from bacterial toxins (20). The classification of adverse reactions to foods is illustrated in Figure 1 (21).

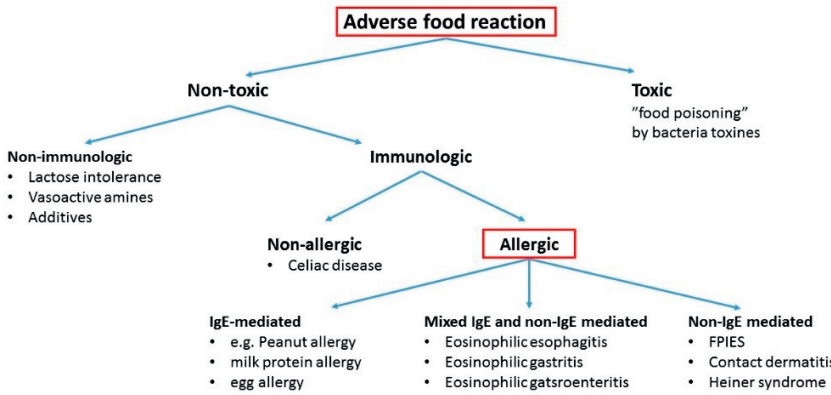


Figure 1. The classification of adverse reactions to foods, adapted from Burks et al (21).

1.2 Prevalence of food allergy

Population surveys, which are mainly performed in children, have concluded that the self-reported adverse reactions after the ingestion of foods are estimated to be between 12-20% (22-24). Current data suggest that food allergy affects more than 1% to 2% but less than 10% of the general population and it still remains unclear whether the prevalence is increasing (25-27). Generally, the prevalence of clinically diagnosed food allergy appears to be about 1.5% to 2% of the adult population and approximately 6% to 8% of children (28). There are also studies that estimate the IgE-sensitization frequency to foods, which report a prevalence for food hypersensitivity at 15 % but a much lower prevalence for IgE-mediated food allergy at only 2 % (29). Data from meta-analysis of different surveys estimate that cow's milk (2.2 %), peanut (1.8 %), and tree nuts (1.7 %) are the most common allergens in children, and shellfish (1.9 %), fruits (1.6 %), and vegetables (1.3 %) are the most common allergens among adults in the United States and Canada (26, 29, 30). Apart from the above, results which are in accordance with data from surveys in Australia and Europe (31-33), there are also surveys from Asia which show that shellfish is the most common causative food allergen while peanut allergy is rare. Among Asian children though, egg and milk allergy are

the most common food allergies with prevalence data comparable to western populations (31-35). Branum et al. observed a trend with increasing prevalence for food allergy in children (36), however, whether there is a similar trend in adults has not been studied. Further, robust data regarding the actual prevalence of adult food allergy is sparse. Therefore, the burden of food allergy in adults could be substantial and reinforces that more data regarding the prevalence of food allergy in adults are needed. In conclusion we do not clearly know the exact prevalence of food allergy in adults (37).

1.3 Etiology, pathogenesis and clinical implications

The course of resolution of food allergy has been well characterized and recently reviewed (30). In general, childhood food allergies to milk, egg, wheat, and soy typically resolve during childhood, whereas allergies to peanut, tree nuts, fish, and shellfish are persistent. Prognosis also varies by disorder; for example, food allergy–related eosinophilic esophagitis (EoE) appears to have a relatively poor chance of resolution. Higher early sIgE levels appear to carry a poorer prognosis than lower values, and decreases in these test results over time might signal resolution (30). There is a complex interplay of environmental influence and genetics that underlie the immunopathogenesis of food allergy and the manifestations of various food-induced allergic disorders. Insights on etiology has mainly been determined from murine models. Several reviews address the role of antigen-presenting cells, T cells, humoral immune responses, homing receptors, signaling pathways, dietary factors, underlying inflammatory states, microbiota, effector cell function, and other aspects of the immune response to dietary antigens. There is limited knowledge though, on why some people become sensitized to different foods and why the prevalence of food allergy seem to have a rising trend (31, 38). A number of possible risk factors have been stated, such as a hygienic life style, industrially processed foods, infections in the early age, the impact of sun exposure and vitamin D deficiency, dietary fat, reduced consumption of anti-oxidants, increased consumption of antacids (reducing digestion of allergens), obesity (steady inflammatory state) and the co-existent effect of other atopic diseases (39-41). The clinical manifestations of food allergy may be symptoms from different organs such as skin (urticaria), respiratory airways (wheezing, bronchial obstruction, sneezing), gastrointestinal tract (acute colic, diarrhea, gases, nausea, vomiting), anaphylaxis with even circulatory collapse, tachycardia, migraine and in some cases arthralgia. It is interesting though to mention that

reports from different population studies show that 40-70% of patients with food allergy report symptoms from the gastrointestinal tract (42).

1.4 Mucosal Immunology

The mucosal epithelial layer in the gut, lungs and genito-urinary tracts represents barriers between the internal and external environments and are an important first line of defense. Immune responses after oral exposure to antigens differ from responses to antigens encountered in tissues, or via skin. The first difference is the local production of large amounts of IgA. Immunoglobulin A is mainly produced in its secretory dimeric form, and is transported through the epithelial cells into the lumen of the GI, respiratory and genito-urinary tracts. The second difference is that oral administration of protein antigens generally induce antigen specific tolerance mediated by regulatory T-cells. The immune defense system associated with the mucosa is referred to as MALT (Mucous Associated Lymphoid Tissue), with BALT for the bronchial tree and GALT for the intestine. The knowledge of mucosal immunity is mainly based on studies of the GI tract and less of the respiratory mucosa. However, it is likely that some features of the immune responses are basically similar in all MALT and that trafficking of immune cells occurs between the different mucosal compartments (43).

1.4.1 The immunity of the gut

The mucosa of the human gut, with a surface area about $300m^2$, acts as a physical barrier to a variety of intraluminal dietary and microbial antigens. The immune system of the gut comprises the major part of the immune cells in the body with about 10^{10} lymphoid cells per meter. This means that the number of lymphocytes in the gut is higher than in the bone marrow, spleen and all the lymph nodes together $2.4x10^{10}$ (43, 44).

The gastrointestinal tract is the largest reservoir of immune cells in the body, and the function of the mucosal immune system is to protect the large surface area of the gastrointestinal tract from invading pathogens and to keep the commensal microbiota compartmentalized. The immune system of the gut is divided into an organized (mesenteric lymph nodes, Peyer's patches and

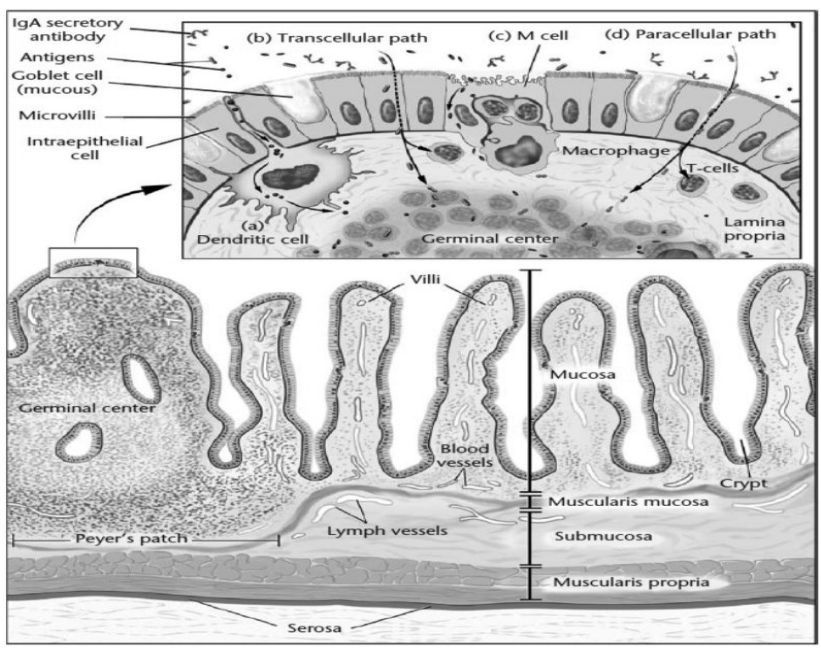
cryptopatches) and a more diffuse (epithelial and lamina propria (LP)) lymphoid compartment. The mucosal immune system is divided from the gut lumen by a single layer of columnar epithelial cells, which secrete a number of factors that contribute to the barrier function, including mucins, antimicrobial peptides, and trefoil factors. The center of each villus, a fingerlike projection in the intestinal wall, contains both a single blind-ended lymphatic vessel termed lacteal and a capillary network. The apical surface of the epithelial cell has numerous tightly packed microvilli covered with a glycocalyx and a thick layer of mucous produced by the interspersed goblet cells. The crypts are invaginations of the epithelium into the underlying tissue that form exocrine glands, that secrete acid, enzymes, water and ions as well as mucous. Each villus is covered by a single layer of epithelial cells, of which about 85% are enterocytes and about 10% are goblet cells (43, 45). The epithelial cells also transport antibodies, particularly IgA, into the intestinal lumen where these antibodies can contribute to barrier function by excluding the uptake of antigens and microbes.

Below the epithelium lies the lamina propria (LP), which is densely populated by resident immune cells, including CD4+ and CD8+ T effector and T regulatory cells, antibody-secreting B cells, and mononuclear phagocytes (macrophages and dendritic cells (DCs). CD4+cells are found in the core of the villus and CD8+ T cells are found close to the epithelium and in between the epithelial cells. The major histocompatibility complex (MHC) class II molecules are found in small vacuoles in the epithelium of the upper third of the villus and in the professional antigen-presenting-cells (APCs) in the LP. The main function of CD8+ cells is believed to be as a memory cytotoxic effector cells, inhibiting virally infected or parasitized epithelial cells (46). The mucosal mast cells are found in the respiratory mucosal surface LP and in the basal LP of the intestine. They arrive to the intestine as immature mast cells from the bone marrow, and differentiate further to mature mast cells, with the help of the cytokines interleukin-3 (IL3), IL-4 and stem cell factor. No mature mucosal mast cells are found in the circulation as they are inhibited to move out from the LP. Eosinophils are also resident cells in the normal intestinal mucosa. These scattered immune cells make up the effector cells of the mucosal immune system, and function to recognize and clear pathogenic challenges from the environment.

The main inductive sites of the GI immune system are the Peyer's patches (PP) and isolated lymphoid follicles (ILFs) that sit directly within the gut mucosa, and the mesenteric lymph nodes (MLN) that drain the whole gastrointestinal tract. The inductive sites are where antigen-specific cellular and humoral immune responses are first generated. A specialized subset of epithelial cells termed M-cells overlies the PP and contributes to the selective uptake of

particulate antigens into this site. In contrast, soluble antigens are primarily taken up across the epithelial lining of the villi and are carried into the MLN. A challenge faced by the mucosal immune system is to discriminate between harmful pathogens and harmless or beneficial commensal organisms as well as food antigens. The lack of reactivity to the commensal flora is in part achieved by a specialized regulatory milieu that also shape the immune response to antigens derived from the diet. Antigen-presenting cells and macrophages of the intestinal mucosa are hypo-responsive to many microbial ligands and secrete high levels of immunoregulatory cytokines such as IL-10. However, the mechanisms responsible for suppression of inappropriate immune reactivity to microbes or food antigens may be quite different (47). The anatomy of the gastrointestinal mucosa is illustrated in Figure 2.

2a



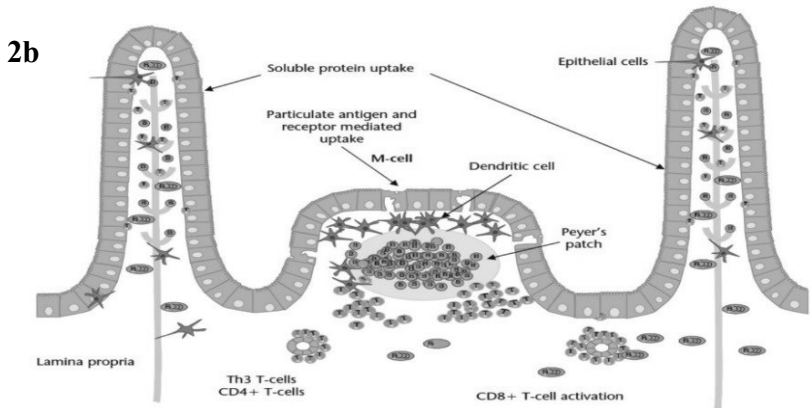


Figure 2a and b. The anatomy of the gastrointestinal mucosa and the sites of antigen uptake in the gut. Antigen taken up by M cells are carried by DCs to the underlying PP where T-cells are activated and helps isotype switching to IgA that occurs in the B-cells. This pathway favors particulate or aggregated antigen. Antigen taken up by intestinal epithelial cells (IECs) normally leads to the induction of regulatory T cells. This pathway favors soluble intestinal antigens. Adapted by Metcalfe et al. (48).

1.5 Mucosal immunology and food allergy

1.5.1 Allergic sensitization

The allergic disease develops when the immune system reacts to a foreign substance, an allergen which in general is harmless. The allergen is usually a protein from an ingested food item or from inhaled pollen or animal dander etc. When the allergen passes the epithelial barrier in the intestine, lung, or skin and encounters the cells of the immune system, a process called sensitization may take place, The allergen is taken up by the antigen presenting cell (APC), which via MHC class II molecule signals to the naïve CD4+ T-cell to develop into a type 2 helper T cell (Th2-cell). The Th2-cell produces cytokines IL-4 and IL-13 and stimulates cognate B-cells to produce allergen-specific Immunoglobulin E (IgE) antibodies. The secreted IgE bind to Fc receptors on tissue bound mast cells and on basophils in the circulation. The mast cells and basophils are coated with specific IgE for which the individual is sensitized. At a following exposure when the same allergen enters the body, the allergen binds to the IgE on the mast cell/basophil, and cross-links the bound IgE, which activates the mast cell or basophil. When activated the cell degranulate and

release inflammatory mediators, such as histamine, proteases and different cytokines, inducing vascular dilatation, smooth muscle contraction, inflammatory cell recruitment and tissue damage. Even in non-allergic individuals the mast cells are coated with IgE, but polyclonal IgE with random specificities and thereby no cross-linking can take place to a specific allergen. It is still not known why some individuals produce IgE when they are exposed to a certain allergen, and others do not. The IgE molecule consists of two identical heavy chains and two identical light chains with one variable region on each chain. The four chains are attached together by disulphide bonds into a Y-shaped molecule. The variable regions make the antibodies capable of binding many different allergens, including macromolecules and chemical compounds, and each antibody clone is specific for one allergen. However, IgE produced against one allergen may bind to other structurally similar allergens with similar epitopes and such a binding is called cross-reactivity (43). The mechanism of food allergen sensitization is illustrated in Figure 3.

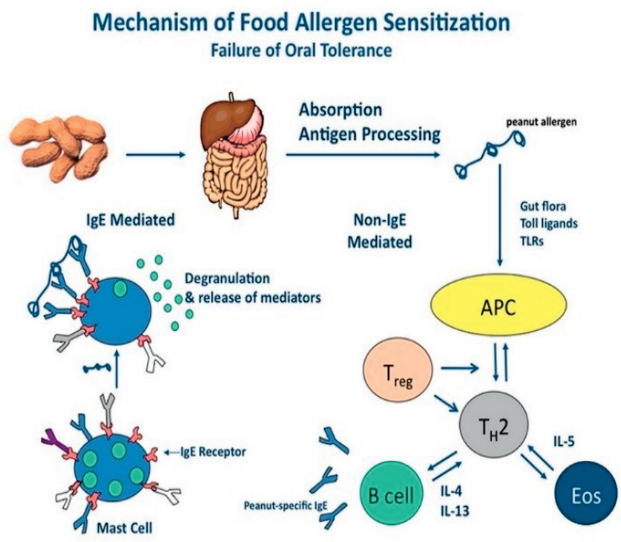


Figure 3. The sensitization process following peanut allergen exposure. Adapted from Otsu K et al(49).

1.5.2 Normal response to foods: mechanisms of oral tolerance

The most important and basic feature of the mucosal immunity is suppression. Suppression is preserved by two phenomena, the controlled or physiologic low grade inflammation and the oral tolerance induction. In lamina propria (LP) there is abundance of plasma cells, T-cells, B-cells, macrophages and DCs. The difference between the LP and a peripheral lymph node is that there is no clear-cut organization in the LP and most of the lymphocytes in the LP are activated memory cells. While the cells remain activated, they do not cause destruction of the tissue or severe inflammation. The phenomenon has been called controlled or physiologic inflammation. The entry and activation of the cells in the LP is antigen driven. The activated T-cells and B-cells released from the MLN express the mucosal integrin $\alpha_4\beta_7$ which recognizes its ligand, MAdCAM, on high endothelial venules in the LP and they then exit the venules and remain activated in the tissue. Bacteria and their products play a role in this persistent state of activation. The failure to produce a pathological state despite the activated state of the lymphocytes is the consequence of suppressor mechanisms that involves regulatory immune cells, cytokines and other cell types. It is well known that LP lymphocytes (LPLs) generally respond poorly when activated via T-cell receptor (TCR). They fail to proliferate although they still produce cytokines and this contributes also to controlled inflammation. However, even in case of an invasive pathogen as e.g. Shigella, the inflammatory response is limited and restoration of the mucosal barrier following eradication of the pathogen is quickly followed by a return to the controlled state and this is thought to be due to suppressor mechanisms (48).

Oral tolerance can be defined as the active, antigen-specific non-response to antigens administered orally. Disruption of oral tolerance results in food allergies and food intolerances such as celiac disease. During digestion, large macromolecules such as proteins, carbohydrates and lipids undergo effective degradation which render the potentially immunogenic substances non-immunogenic. In case of proteins, digestive enzymes break down large polypeptides into non-immunogenic di- and tri-peptides, too small to bind to major histocompatibility complex (MCH) molecules. However, several groups have reported that up to 2% of dietary proteins enter the draining enteric vasculature (50), and the answer to the critical question about how we normally regulate the response to these antigens is by the induction of antigen specific oral tolerance.

The mechanisms of oral tolerance are complex and depend on several factors. These factors are:

1. The age of the host: The reduced tolerance in the neonates, which may be related to the rather permeable barrier that exists in the newborn and/or the immaturity of the mucosal immune system. The limited diet of the newborn may serve to protect the infant from generating a significant response to food antigens
2. The genetics of the host, and the nature and form of antigen: From studies in mice it was shown that some strains are easily tolerized to a certain antigen while others are not. It is suggested that the nature and form of the antigen play a significant role in the tolerance induction. Protein antigens are the most tolerogenic while carbohydrate and lipids are much less effective in inducing tolerance. Insolubility and aggregation may also render a luminal antigen incapable of being sampled. Lastly, prior sensitization to an antigen via extra-intestinal routes, may hamper the development oral tolerance.
3. Dose of the antigen: Low doses of antigen appear to activate regulatory/suppressor T-cells. Th3 cells were the initially described regulatory/suppressor cells in oral tolerance (51). These cells secrete transforming growth factor-β (TGF- β), which is a potent suppressor of T- and B-cell responses while promoting the production of IgA. The production of TGF-β by Th3 cells elicited by low-dose antigen administration, helps to explain an associated phenomenon of oral tolerance, bystander suppression. If a second antigen is co-administered systemically with the tolerogen, suppression of T- and B-cell responses to that antigen will also occur. Experiments in mice have shown that reduced IL-10 production in PPs may contribute to the development of food allergies (52) while increased numbers of CD4+, CD25+ T-cells expressing cytotoxic T-lymphocyte antigen 4 and cytokines TGF-β and IL-10 are associated with oral tolerance. Furthermore, tolerance studies in mice depleted of CD25+ T cells along with TGF- β neutralization inhibited the induction of oral tolerance both by high and low doses of oral antigen, suggesting that CD4+CD25+ T-cells and TGF-β together are involved in the induction of oral tolerance. Markers such as glucocorticoid-induced TNF receptor and the transcription factor FoxP3 have been shown to be preferentially expressed by CD4+CD25+Tregs (53). Deficiency or non-functional FoxP3 results in autoimmune, inflammatory and multi-allergy syndrome in both humans (IPEX) and mice (Scurfy) (54). Although it has been clearly demonstrated that tolerance is mediated by Tregs, it is not yet understood if the lack of clinical reactivity to all normal dietary antigens mediated by Tregs. In mice and humans, a lack of Foxp3+ T cells leads to enteropathy, eczema, and elevated IgE. Severe food allergy may occur as one manifestation of Foxp3 mutations. Mice having a defect in induced Foxp3+ Tregs, with

normal levels of thymically-derived natural Tregs, exhibit a Th2-skewed mucosal inflammation and generation of an inflammatory antibody response. These data show that Tregs have a role in the suppression of responses to mucosally-derived antigens. Children who have outgrown their milk allergy have an increased frequency of circulating CD4+ CD25+ Tregs after an oral milk challenge and a reduced proliferation of milk-specific T cells (55). The depletion of $CD4^{+}CD25^{+}$ Tregs though, restores the (in vitro) proliferative response in milk-tolerant subjects. These data suggest that Tregs may be involved in the development of clinical tolerance to food allergens. However, the presence of food antigen-specific Tregs has not yet been demonstrated in healthy human subjects (47).

4. State of the barrier: Several states of barrier dysfunction are associated with aggressive inflammation and a lack of tolerance. Increased permeability throughout the intestine has been shown in animal models of anaphylaxis where antigens are able to pass through paracellular spaces by the disruption of tight junctions. It is speculated that barrier disruption leads to altered mucosal sampling and escape from regulatory pathways. Here the processing of protein antigens by the IEC may be of crucial importance for a tolerogenic response.

Oral tolerance has been demonstrated in humans although its efficacy is not elucidated. One clear difference between humans and mice is that tolerance is induced for T-cells but less so for B-cells (56). This difference may have relevance in human antibody-mediated diseases (48).

1.5.3 Gastrointestinal antigen-trafficking cells

Probably the best defined pathway of antigen trafficking is in the GI-tract through the specialized epithelium overlying the organized lymphoid tissue of the GALT; the PP. This specialized epithelium contains M-cell as stated before. The M-cell is unique in contrast to adjacent absorptive epithelium because it has few microvilli, a limited mucin overlayer, a thin elongated cytoplasm and a shape that forms a pocket around subepithelial lymphocytes, macrophages and DCs. The M-cell is a conduit to the PP and therefore antigens transcytosed across the M-cell and into the subepithelial pocket are taken up by macrophages/DCs and carried into the PP. Once in the patch, they instruct T-cells that after activation leave the patch together with cognate B cells and

migrate to the mesenteric lymph node where they proliferate and differentiate. Eventually the T and B cells leave the MLN and migrate back to seed the mucosal sites and the B cells undergo terminal maturation into IgA producing plasma cells. Several groups have suggested that M-cells are involved in tolerance induction as well, but there are some problems with this implication. First, M-cells are more limited in their distribution, so that antigen sampling by these cells may be modest in the context of the whole gut. Second, M-cells are rather inefficient at taking up soluble proteins which are the best tolerogens as stated earlier. More recent data on mouse models though demonstrate that tolerance can occur in the absence of M-cells and PPs (57), which suggest that PPs and M cells are not of importance for the induction of oral tolerance (48).

DCs play an important role in tolerance and immunity of the gut. The function of these APCs, help in maintaining gut integrity through expression of tight junction proteins, and to orchestrate T cell responses. DCs continuously migrate within lymphoid tissues even in the absence of inflammation and present self-antigens, e.g. from dying apoptotic cells, to maintain self-tolerance. DCs process internalized antigens slower than macrophages, allowing for adequate accumulation, processing, and eventually presentation of antigens. They are found within the LP and their presence is dependent on the chemokine receptor CX3CRI. Studies are ongoing to determine the chemokines that are responsible for migration of DCs to the LP. However, what has been found is that epithelial cells express CCL25, which is the ligand for CCR9 and CCR10 and may thus be a DC chemokine in the small bowel, and CCL28, a ligand for CCR3 and CCR10 may be a DC chemokine in the colon (58). Several studies have examined the pathway by which DCs maybe tolerogenic including their maturation status at the time of antigen presentation to T-cells; downregulation of costimulatory molecules CD80 and CD86, production of suppressive cytokines IL10, TGF-β and their production of all-trans retinoic acid. This suggests that dysregulation of DCs, systemic and gut derived, influences the development of food allergy and is necessary for controlling immune responses (59, 60).

The other cell type potentially involved in antigen sampling is the absorptive epithelium. These cells not only take up soluble proteins but they also express MHC class I and II. The capacity of intestinal epithelial cells (IECs) to serve as APC to both CD4+ and CD8+ cells and the importance of INF-γ as well as MCH class II in the induction of tolerance in has been previously demonstrated (61, 62). Epithelial cells may interact both with IELs (CD8+ T cells in close contact with the epithelium) or LPLs.

How all this is contributing to the process of food allergy and whether allergens traffic differently in predisposed individuals are questions that still need to be studied further. The real key questions is why tolerance mechanisms dissolve occasionally and how the initial IgE is produced. The answer to these questions will provide major insights into the pathogenesis of food allergy (48).

1.6 Overview of the clinical gastrointestinal food allergy

Different population studies have shown that about 20-30% of people experience some form of food intolerance but in only 10-20% of these subjects could it be proven that they suffer from intolerance to a specific food item. When these subjects are investigated with double blind placebo controlled food challenges (DBPCFC), food intolerance could only be verified in about 2% of these patients (63). Food intolerance may have both non-immunological and immunological entities. Immunological food reactions may be caused by IgE-mediated, non-IgE-mediated or mixed mechanisms. The symptomatology of food allergies or intolerances may co-exist with other gastrointestinal pathological conditions which make the clinical investigation complex. About 40-70% of patients with food allergy report gastrointestinal symptoms (42). An interesting relation between patients with food intolerance and IBS has been documented. In previous studies it has been shown that more than 60% of patients with IBS experience worsening of the gastrointestinal symptoms after the ingestion of foods rich in carbohydrates and fat, alcohol, coffee, spices and even food rich in biogenic amines or a histamine-release effect (64, 65). The connection between atopy and IBS has also been documented in a study where patients with IBS and asthma, allergy or eczema showed a greater intestinal permeability compared to a non-atopic control group (66). Patients with IBS-like symptoms also experience worsening of their gastrointestinal symptoms during the pollen season (67). The novelty, that even subjects with birch pollen allergy, but without any gastrointestinal symptomatology, show signs of allergic inflammation in the intestinal mucosa during the pollen season is presented in the first study of this thesis (68). Eosinophilic esophagitis (EoE) is another pathologic condition with very heterogeneous pathophysiology that is quite often related to atopic manifestations. It has been well documented that EoE may be related to pollen allergy since esophageal biopsies have revealed a clear eosinophilic infiltration in the esophagus during the pollen season (69) and that most cases of EoE are diagnosed during the pollen season (70). EoE can be triggered by food antigens independently of the type of underlying

immunological mechanism. For these reasons, clinical investigation of an underlying sensitization to foods is recommended with the accompanied elimination diet and control esophagoscopies during the follow-up (71).

In many cases when investigating gastrointestinal food allergy though a non-IgE-mediated mechanism can be verified by the conventional allergy tests, but it is difficult to state, whether this may be due to low sensitivity of the tests regarding GI-allergy or that the underlying immune mechanism is other than IgE-mediated.

The term "entopy" has been used to describe patients with a local allergic response in non-atopic subjects with local production of IgE in the mucosa (72). Entopic IgE-production has been described in patients with nasal polyps (73), chronic and non-atopic idiopathic sinusitis (74, 75) as well as in non-allergic asthma (76). Local allergic inflammation in the duodenal biopsies of food hypersensitivity adults despite the lack of specific IgE, has been presented by Lin et al. in food allergic subjects verified by DBPCFC (77). as well as by Bengtsson et al. who showed increased concentrations of histamine and eosinophilic cationic protein in the gut of patients after challenge with cow's milk (78). A few other studies describes a local edema in the small intestinal mucosa after challenge with the symptom giving food in subjects who lacks systemic specific IgE (79, 80). Altered intestinal barrier function and increase in the intestinal permeability were found to be associated with food allergy as well as with celiac disease, inflammatory bowel disease and type I diabetes (81). It is stated that an increase in the intestinal permeability may be caused by sensitization of the epithelium and after degranulation of mast cells (82). Increase in the intestinal permeability has also been observed in patients with bronchial asthma, atopic eczema and chronic urticaria (83-85). In addition, increased concentrations of hyaluronan and albumin in the jejunal mucosa, which reflect mucosal edema with consequent increased intestinal permeability, has been described in cow's milk allergic patients when challenged with cow's milk (86).

Finally, sporadic reports suggest that food allergy may even have co-existent extra-intestinal manifestations as joint swelling, arthralgia or headache for which more evidence and research is needed though (87, 88). In case of rheumatoid arthritis, it has been suggested from several case studies that elimination of cereals as well as cow's milk has led to significant or marked improvement of disease symptoms (89-94). Sporadic reports even suggested remission of the disease when eliminating cereals from the diet (95).

1.7 Food allergy and anaphylaxis

Food-induced anaphylaxis accounts for about 30-50% of anaphylaxis cases in the emergency departments in North America, Europe and Australia (96). The mechanism of food-induced anaphylaxis may be immunologic (IgE-mediated or food-exercise-induced) or non-immunologic (vasoactive amines, additives or conditions resembling FPIES). The most common foods causing food anaphylaxis in children living in western countries are milk, egg, peanut and other tree-nuts while in adults shellfish, peanuts and other tree-nuts dominates (97). The prevalence of food-induced anaphylaxis in western countries is increasing, but it is difficult to be determined due to limitations concerning the definition of the diagnosis and the various methodologies used in the different studies (27). In case of anaphylaxis the skin symptoms is seen in most of the cases 80-90% followed by the airways 70%, gastrointestinal tract 40%, syncope/dizziness 35% and hypotension 10%, while obstruction of the airways is reported to be the most common cause of death (98, 99). Symptoms from the gastrointestinal tract, the airways and the skin are most prominent in children and teenagers while cardiovascular symptoms and chock occur more often in adults (100). The course of food-induced anaphylaxis may be accompanied by a biphasic reactions in almost 20% of the cases during 4-8h from the onset of the first reaction (101, 102). FDEIA is a special type of reaction in which an individual who is IgE-sensitized to a food item may react with anaphylaxis if the ingestion of this food is accompanied by physical exercise. Until now, this phenomenon is well documented for wheat but it may also occur for other foods. In some cases, the combination of the ingestion of the offending food with physical exercise, and the presence of co-factors such as of drugs (acetylacilic acid, NSAID), alcohol, on-going infection, sudden temperature changes, menstruation or high titers of pollen are needed to precipitate the anaphylactic reaction (103). A relatively newly described delayed anaphylaxis due to ingestion of red meat is documented and may occur 3-7h after the ingestion. This may occur due to an IgE-antibody response to a carbohydrate structure in the red meat (alpha-gal). Current research has shown that tick-bites may induce the production of IgE-antibodies to alpha-gal (104, 105).

The deficiency in diaminoxidase enzyme (DAO) (which degrades histamine) may be the cause of anaphylaxis after the ingestion of food rich in biogenic amines (106). Yellow, orange and red colorants in different foods together with some thickening preservatives and sulfites may also cause additive-induced anaphylaxis from different foods (18).

1.8 Peanut allergy

Peanut allergy is one of the leading clinical problems in the field of food allergy due to high risk for anaphylaxis in cases with severe allergy. Clinical peanut allergy has been reported in 0.2-1.8% of children and has increased in westernized countries during the last decades (107, 108). The increase may be due to altered dietary habits in previously unexposed populations coupled with changes in peanut processing procedures resulting in increased allergenicity of peanut antigens, but the issue still remains unresolved. In adults the prevalence of peanut allergy is estimated at 0.6% (26). The prevalence of peanut sensitization is about 3%-6%, and the reactions may be severe (100) with negative impact on quality of life (109). In many countries peanut sensitized patients have been advised to strictly avoid peanuts if they have experienced allergic symptoms. The processing of the peanut has been proposed to play role in its allergenicity. Roasted peanut components bind to IgE from peanut allergic patients to a higher degree than raw peanut from the same cultivars (110). Peanut processing, cooking and frying seems to reduce allerginicity in the peanut proteins (111). Consequently, in Asian countries where cooked peanuts are mainly served, allergy is rarely reported (110).

1.9 Diagnostic methods for food allergy in general and peanut allergy in particular

The most important diagnostic tool in the investigation of food allergy comprises a detailed and careful clinical history of the patient's symptoms from different foods and a physical examination. However, this detective like clinical investigation cannot be used alone for elucidating the etiology of the patient's food intolerances and therefore it should always be combined with conventional allergy tests (skin prick test and IgE-tests in the blood), along with elimination and challenge trials sometimes in combination with other complementary diagnostic tools.

1.9.1 Skin prick testing and serum IgE analysis

Skin prick test (SPT) was first introduced in 1942 and is still used widely in the diagnosis of allergy. SPT is used for a more direct estimation to confirm if there is an IgE response to the allergen or not together with the first clinical

examination. In children the wheal diameter at SPT correlates rather well with the likelihood of clinical food allergy (112-115), which is not always the case in in adult patients (116). The SPT results are sensitive to variations in the testing circumstances such as skin reactivity, skills of the person performing the test or ethnicity of the patient (117-120). The atopy-patch tests have been of value in the diagnosis of food intolerances in children with atopic dermatitis (121, 122) but its use has limitations when applied in teen-agers and adults (123). The first commercial serum IgE test, the radio absorbent test (RAST), which utilized solid phase allergens incubated with patient's sera was introduced in 1972 (9). Bound IgE was detected with radio-isotopically labeled anti-IgE reagent and radioactivity was measured by a gamma counter (124). There are several commercial IgE assays available today and IgE results are normally expressed as kU/L. In Sweden, most IgE assays are performed by ImmunoCap test (Thermo Fischer Scientific, Uppsala). Specific allergen IgE-level thresholds were established for the first time in the pediatric population by Sampson et al in 2001 for the major food allergens wheat, milk, egg, fish, soy and peanut (125). Concerning peanut, the 95% decision point for peanut extract was set previously to 15 kU_A/L (125). Similar probability curves concerning cut-off IgE levels for different foods were also supported by other studies (126, 127). However it is important to mention that these cut-off levels have their limitations, and should only be applied in homogenous patient populations. This means that they apply mainly to children since most of the studies were performed in the pediatric population (128). Neither skin prick tests nor IgE-tests are diagnostic for **all** patients and their efficiency varies depending on the type of food (129, 130). It is also worth mentioning that in many cases there is a disagreement between SPT and IgE-tests in diagnosing allergic sensitization (131).

1.9.2 Molecular allergology

SPT and allergen-specific tests can usually not resolve the matter why the same specific IgE (sIgE) antibody can bind to proteins with similar structures present in different allergen sources. In allergy diagnostics only a limited number of allergens are routinely assayed with SPT and sIgE. Performing a large number of SPT may be disagreeable for the patient and a laboratory testing of a large number of sIgE can be both expensive and demands a large blood sample (132). The component resolved diagnostics (CRD) approach (133) has been developed for testing IgE reactivity to highly purified and recombinant allergens (134). The allergens are attached to a solid phase micro array, and allows simultaneous analysis and monitoring of patient-specific antibody

profiles for a large variety of allergens in a single analytical step (135) This development allows simultaneous analysis and has enabled the identification of protein families and cross-reactivity-patterns of importance in allergy (136) as well as monitoring of patient specific antibody profiles in a single analytical step (135). ImmunoCAP ISAC® (Thermo Fischer Scientific, Uppsala, Sweden) is the first in vitro diagnostic tool based on this biochip technology. It is a miniaturized immunoassay platform that allows multiplex measurement of specific IgE antibodies for 112 natural purified and recombinant allergen molecules using only 30μl of serum or plasma (137). In a two-step assay, IgE antibodies from the patient's serum are allowed to bind to allergen components on the chip, and after a short washing step, the allergen-bound IgE antibodies are detected by a fluorescence-labeled anti-IgE antibody. The test results are measured with a biochip scanner and evaluated using a dedicated software. ImmuCAP ISAC® is a semiquantitative test and results are reported in ISAC Standardized Units (ISU). The extract allergen preparations (used in SPT, for sIgE detection and classic immunotherapy) are a mixture of several different molecules together with the specific allergenic proteins along with pan-allergens (molecules that are present also in mixtures from related sources with highly homologous molecules) and finally cross-reacting allergens (molecules that displays a certain degree of homology in the tertial structure of the protein) (138). These molecules are defined as components and the mixture of different components constitutes an allergen. A specific component is in general responsible for the primary (genuine) sensitization to that allergen, whereas activity to pan-allergens or cross-reacting allergens are secondary phenomena, but can still play a role for the symptoms seen in a clinical allergy.

Components are available for in vitro diagnosis in two different forms: as recombinant allergens and as highly purified molecules (138). Components are named using a conventional notation. Consequently, the first 3 letters e.g. Bet correspond to the first 3 letters of the Linnean family name of the source, in this case Betula. The fourth letter indicates the first letter the species e.g. v for verrucosa followed by a number that indicates a specific protein from that source. Thus, a common allergenic component from the European birch is named nBet v 1. Finally the prefix "r" or "n" of the component name is indicative of its origin, which can be either recombinant or natural. In general IgE antibodies directed to a specific component is suggestive of genuine sensitization if the clinical symptoms strongly associate that allergen to the patients history (139). CRD offers possibilities that are not available with extract based standard techniques such as SPT and sIgE. CRD can effectively help in distinguishing between primary sensitization and cross-reactivity in patients with suspected multi-sensitization to various allergens (134). This may have a significant impact on the patient management in terms of risk assessment, advice to avoid allergens, patient selection for immunotherapy and

immunotherapy regime (140). ImmunoCAP ISAC is a particularly powerful diagnostic tool in poly-sensitized patients not only to detect the actual molecular component involved in the allergy but also to rule out cross-reacting allergens and other components (such as LTP), which may be responsible for the observed symptoms. In some cases of unexplained anaphylaxis ImmunoCAP ISAC could be used as a complementary diagnostic tool in order to exclude possible hidden causative allergen from multiple allergen sources. Microarray IgE assay inarguably represents an advancement in allergy diagnosis as a third-level approach in poly sensitized subjects, when the traditional diagnosis may be problematic. Finally, when information on reactivity to many single recombinant allergens is required to define an accurate sensitization profile, ISAC is preferable in terms of costs and efficiency (141). However, it is important to state that ISAC results should be assessed by a competent allergologist in terms of setting the correct allergy diagnosis.

Allergen components can be classified as belonging to different protein families, according to their function and structure as following (136):

1. Non-specific lipid transfer proteins (nLTP): stable to heat and digestion causing reactions also to cooked foods and are often associated with systemic and more severe reactions in addition to OAS and with allergic reactions to fruit and vegetables in southern Europe.
2. Storage proteins: found in seeds and serves as source material during the growth of a new plant, often stable and heat-resistant proteins causing reaction also to cooked foods (2S albumins, 7S albumins, 11S albumins, gliadins).
3. Pathogenesis-related protein family 10 proteins (PR10-proteins): They are Bet v 1 homologues and often associated with local symptoms such as OAS and with allergic reactions to fruit and vegetables in northern Europe and may predispose reactions to Rosaceae fruits hazelnut, carrot and celery.
4. Profilins: actin-binding proteins showing great homology and cross-reactivity even between distant related species which are seldom associated with clinical symptoms but may cause demonstrable or even severe reactions in a small minority of patients.
5. Cross-reactive carbohydrate determinants (CCD): can be used as a marker for sensitization to protein carbohydrate moieties (pollen, hymenoptera etc.) and these are seldom associated with clinical symptoms but may cause adverse reactions in a limited number of patients.
6. Calcium-binding proteins: highly cross-reactive proteins present in most pollens but not in plant foods.

7. Serum albumins: common proteins present in different biological fluids and solids e.g. cow's milk, beef, egg and chicken, sensitization may give rise to airway reactions to mammalian animals as food reactions to meat and milk.
8. Parvalbumins: major allergens in fish and a marker of cross-reactivity among different species of fish and amphibians which are stable to heat and digestion.
9. Tropomyosins: actin-binding proteins in muscle fibers which may be used for cross-reactivity between crustaceans, mites, cockroach and nematodes.
10. Lipocains: stable proteins and important allergens in animals which display only limited cross-reactivity between species.

The complete list of allergen components included in ImmunoCAP ISAC® are presented in Figure 4.

4a **ImmunoCAP ISAC 112 Allergen Components, con't**

Allergen component	Allergen source COMMON NAME	LATIN NAME	PROTEIN GROUP
Food Allergens			
rAra h 1	Peanut	*Arachis hypogaea*	Storage protein ,7S globulin
rAra h 2	Peanut	*Arachis hypogaea*	Storage protein, Conglutin
rAra h 3	Peanut	*Arachis hypogaea*	Storage protein, 11S globulin
nAra h 6	Peanut	*Arachis hypogaea*	Storage protein, Conglutin
rAra h 8	Peanut	*Arachis hypogaea*	PR-10 protein
rAra h 9	Peanut	*Arachis hypogaea*	Lipid transfer protein (nsLTP)
rGly m 4	Soybean	*Glycine max*	PR-10 protein
nGly m 5	Soybean	*Glycine max*	Storage protein, Beta-conglycinin
nGly m 6	Soybean	*Glycine max*	Storage protein, Glycinin
nFag e 2	Buckwheat	*Fagopyrum esculentum*	Storage protein, 2S albumin
rTri a 14	Wheat	*Triticum aestivum*	Lipid transfer protein (nsLTP)
rTri a 19.0101	Wheat	*Triticum aestivum*	Omega-5 gliadin
nTri a aA_TI	Wheat	*Triticum aestivum*	
nAct d 1	Kiwi	*Actinidia deliciosa*	
nAct d 2	Kiwi	*Actinidia deliciosa*	Thaumatine-like protein
nAct d 5	Kiwi	*Actinidia deliciosa*	
rAct d 8	Kiwi	*Actinidia deliciosa*	PR-10 protein
rApi g 1	Celery	*Apium graveolens*	PR-10 protein
rMal d 1	Apple	*Malus domestica*	PR-10 protein
rPru p 1	Peach	*Prunus persica*	PR-10 protein
rPru p 3	Peach	*Prunus persica*	Lipid transfer protein (nsLTP)
Aeroallergens			
nCyn d 1	Bermuda grass	*Cynodon dactylon*	Grass group 1
rPhl p 1	Timothy	*Phleum pratense*	Grass group 1
rPhl p 2	Timothy	*Phleum pratense*	Grass group 2
nPhl p 4	Timothy	*Phleum pratense*	
rPhl p 5	Timothy	*Phleum pratense*	Grass group 5
rPhl p 6	Timothy	*Phleum pratense*	
rPhl p 7	Timothy	*Phleum pratense*	Polcalcin
rPhl p 11	Timothy	*Phleum pratense*	
rPhl p 12	Timothy	*Phleum pratense*	Profilin
rAln g 1	Alder	*Alnus glutinosa*	PR-10 protein
rBet v 1	Birch	*Betula verrucosa*	PR-10 protein
rBet v 2	Birch	*Betula verrucosa*	Profilin
rBet v 4	Birch	*Betula verrucosa*	Polcalcin
rCor a 1.0101	Hazel pollen	*Corylus avellana*	PR-10 protein
nCry j 1	Japanese ceder	*Cryptomeria japonica*	
nCup a 1	Cypress	*Cupressus arizonica*	
nOle e 1	Olive	*Olea europaea*	
nOle e 7	Olive	*Olea europaea*	Lipid transfer protein (nsLTP)
rOle e 9	Olive	*Olea europaea*	
rPla a 1	Plane tree	*Platanus acerifolia*	
nPla a 2	Plane tree	*Platanus acerifolia*	
rPla a 3	Plane tree	*Platanus acerifolia*	Lipid transfer protein (nsLTP)
nAmb a 1	Ragweed	*Ambrosia artemisiifolia*	
nArt v 1	Mugwort	*Artemisia vulgaris*	
nArt v 3	Mugwort	*Artemisia vulgaris*	Lipid transfer protein (nsLTP)
rChe a 1	Goosefoot	*Chenopodium album*	
rMer a 1	Annual mercury	*Mercurialis annua*	Profilin
rPar j 2	Wall pellitory	*Parietaria judaica*	Lipid transfer protein (nsLTP)
rPla l 1	Plantain (English)	*Plantago lanceolata*	
nSal k 1	Saltwort	*Salsola kali*	

4b **ImmunoCAP ISAC 112 Allergen Components, con't**

Allergen component	Allergen source COMMON NAME	LATIN NAME	PROTEIN GROUP
Aeroallergens			
rCan f 1	Dog	*Canis familiaris*	Lipocalin
rCan f 2	Dog	*Canis familiaris*	Lipocalin
nCan f 3	Dog	*Canis familiaris*	Serum albumin
rCan f 5	Dog	*Canis familiaris*	Arginine esterase
rEqu c 1	Horse	*Equus caballus*	Lipocalin
nEqu c 3	Horse	*Equus caballus*	Serum albumin
rFel d 1	Cat	*Felis domesticus*	Uteroglobin
nFel d 2	Cat	*Felis domesticus*	Serum albumin
rFel d 4	Cat	*Felis domesticus*	Lipocalin
nMus m 1	Mouse	*Mus musculus*	Lipocalin
rAlt a 1	Alternaria	*Alternaria alternata*	
rAlt a 6	Alternaria	*Alternaria alternata*	Enolase
rAsp f 1	Aspergillus	*Aspergillus fumigatus*	
rAsp f 3	Aspergillus	*Aspergillus fumigatus*	
rAsp f 6	Aspergillus	*Aspergillus fumigatus*	Mn superoxide dismutase
rCla h 8	Cladosporium	*Cladosporium herbarum*	
rBlo t 5	House dust mite	*Blomia tropicalis*	
nDer f 1	House dust mite	*Dermatophagoides farinae*	
rDer f 2	House dust mite	*Dermatophagoides farinae*	
nDer p 1	House dust mite	*Dermatophagoides pteronyssinus*	
rDer p 2	House dust mite	*Dermatophagoides pteronyssinus*	
rDer p 10	House dust mite	*Dermatophagoides pteronyssinus*	Tropomyosin
rLep d 2	Storage mite	*Lepidoglyphus destructor*	
rBla g 1	Cockroach	*Blattella germanica*	
rBla g 2	Cockroach	*Blattella germanica*	
rBla g 5	Cockroach	*Blattella germanica*	
nBla g 7	Cockroach	*Blattella germanica*	Tropomyosin
Other			
rApi m 1	Honey bee venom	*Apis mellifera*	Phospholipase A2
nApi m 4	Honey bee venom	*Apis mellifera*	Melittin
rPol d 5	Paper wasp venom	*Polistes dominulus*	Venom, Antigen 5
rVes v 5	Common wasp venom	*Vespula vulgaris*	Venom, Antigen 5
rAni s 1	Anisakis	*Anisakis simplex*	
rAni s 3	Anisakis	*Anisakis simplex*	Tropomyosin
rHev b 1	Latex	*Hevea brasiliensis*	
rHev b 3	Latex	*Hevea brasiliensis*	
rHev b 5	Latex	*Hevea brasiliensis*	
rHev b 6.01	Latex	*Hevea brasiliensis*	
rHev b 8	Latex	*Hevea brasiliensis*	Profilin
nMUXF3	Sugar epitope from Bromelain		CCD-marker

4c

ImmunoCAP ISAC 112 Allergen Components			
Allergen component	Allergen source COMMON NAME	LATIN NAME	PROTEIN GROUP
Food Allergens			
nGal d 1	Egg white	*Gallus domesticus*	Ovomucoid
nGal d 2	Egg white	*Gallus domesticus*	Ovalbumin
nGal d 3	Egg white	*Gallus domesticus*	Conalbumin/Ovotransferrin
nGal d 5	Egg yolk/chicken meat	*Gallus domesticus*	Livetin/Serum albumin
nBos d 4	Cow's milk	*Bos domesticus*	Alpha-lactalbumin
nBos d 5	Cow's milk	*Bos domesticus*	Beta-lactoglobulin
nBos d 6	Cow's milk and meat	*Bos domesticus*	Serum albumin
nBos d 8	Cow's milk	*Bos domesticus*	Casein
nBos d lactoferrin	Cow's milk	*Bos domesticus*	Transferrin
rGad c 1	Cod	*Gadus callarias*	Parvalbumin
nPen m 1	Shrimp	*Penaeus monodon*	Tropomyosin
nPen m 2	Shrimp	*Penaeus monodon*	Arginine kinase
nPen m 4	Shrimp	*Penaeus monodon*	Sarcoplasmic Ca-binding protein
rAna o 2	Cashew nut	*Anacardium occidentale*	Storage protein, 11S globulin
rBer e 1	Brazil nut	*Bertholletia excelsa*	Storage protein, 2S albumin
rCor a 1.0401	Hazelnut	*Corylus avellana*	PR-10 protein
rCor a 8	Hazelnut	*Corylus avellana*	Lipid transfer protein (nsLTP)
nCor a 9	Hazelnut	*Corylus avellana*	Storage protein, 11S globulin
nJug r 1	Walnut	*Juglans regia*	Storage protein, 2S albumin
nJug r 2	Walnut	*Juglans regia*	Storage protein, 7S globulin
nJug r 3	Walnut	*Juglans regia*	Lipid transfer protein (nsLTP)
nSes i 1	Sesame seed	*Sesamum indicum*	Storage protein, 2S albumin

Figure 4a, b and c: The allergen components included in the latest version of ImmunoCAP ISAC® (Thermo Fischer Scientific, Uppsala, Sweden).

1.9.3 Molecular Allergology of peanut

Peanuts are the seeds of the peanut plant (*Arachis hypogaea*) which is a member of the legume family (Fabaceae). The peanut is botanically related to beans and peas but not to tree nuts. Peanut seeds are currently widely used as food source in most countries of the world due to their high quality protein and oil content. Currently, the IUIS allergen nomenclature subcommittee accepts 12 peanut allergens (Ara h 1,2,3,5,6,7,8,9,10,11,12 and 13). Two allergens belong to the cupin (Ara h 1 and Ara h 3) and four to the prolamin (Ara h 2, 6, 7 and Ara h 9) superfamily, and six are distributed among the profilins (Ara h 5), Bet v 1-like proteins (Ara h 8), oleosins (Ara h 10 and Ara h 11), and defensins (Ara h 12 and Ara h 13). Peanuts contain 44–56 % oil and 22–30 % protein (142). The cupin Ara h 1 was determined to contribute to 12–16 %, and the 2S albumin Ara h 2 to 5.9–9.3 % of the total protein content of a peanut (143). In a recent study, all known peanut allergen classes were determined to comprise 85 % of the total protein content of a peanut while Ara h 1, Ara h 2, and Ara h 3 together accounted for 75 % (144). Clinically, the use of Ara h 1,2,3,6 and Ara h 8 for the diagnosis of peanut allergy or for generating a peanut sensitization profile, is currently best documented (145-148). Recently, it was shown that rAra h 2 was found to be the best predictor of clinical peanut allergy in 28% of adult patients, but rAra h 2 alone, could neither discriminate between mild or severe peanut allergy nor to exclude peanut allergy (149). Sensitization to Ara h 9 with a clinical impact is mostly documented in southern Europe (150). In northern Europe though, sensitization to Ara h 8, which is homologue to Bet v 1 from birch pollen, and which indicate cross-reactivity between birch and peanut sensitization, is observed more often in allergic or sensitized patients to peanuts compared to patients from the southern regions of Europe (148). In peanut-allergic patients a clinically relevant co-sensitization to other legumes such as soybean, lupin, lentil, or pea may occur, however, little information is available about the clinical impact of this cross-reaction. In a group of 39 peanut-sensitized patients, 82, 55, and 87 % of patients were also sensitized to lupine, pea, and soybean, respectively, but based on DBPCFC only 29–35 % had symptoms after ingestion of these beans (151). In addition, between 20 and 40 % of peanut-allergic individuals also have a co-existing allergy to other related tree nuts (152, 153). In a large study including 324 peanut-allergic patients, 86 % were sensitized to tree nuts, and 34 % had a clinical documented allergy to tree nuts. (154). The number of allergics to other nuts may even be higher since up to 60 % of adult peanut-allergic individuals were found to be allergic to one or more tree nuts with the most common reactivity of 49 % to hazelnuts (155). From several studies on children, IgE to rAra h 2 was assessed to be the most important reactivity that suggested severe allergy to peanut if the concentration exceeds 0.35 kU/L in

ImmunoCap analyses. From a study, which was done in a mixed population of children and adults Eller and Bindslev-Jensen, suggested a value of >1.63 kU/L for rAra h 2 to predict severe allergy to peanut , with a specificity 100% and sensitivity 70%, (156). Recently Beyer et al found that a 90% probability for a positive peanut challenge was reached at 14.4 kU/L of IgE to rAra h 2 (157). As illustrated by these results there is still no consensus yet concerning specific cut-off values for IgE to rAra h 2 for predicting severe peanut allergy.

1.9.4 Oral food challenges

Food challenges are widely used in the investigation of food allergy since the diagnosis cannot always be set based on the patient history in combination with SPT and IgE-tests. Food challenges should be performed either to confirm or to exclude the diagnosis of food allergy or to determine the sensitivity of the patient to the offending food (threshold value), according to the EAACI position paper (158). Food challenges can be performed as an open oral food challenge (OFC) or double-blind placebo controlled food challenge (DBPCFC). In DBPCFC neither the patient nor the doctor or the nurse know if the food challenge is performed with the food item or with placebo. DBPCFC is considered as the “gold standard” method in the diagnosis of food intolerance although it does not reveal the etiology mechanism. It is important though to mention that in some cases it is difficult to interpret the patient’s symptoms caused either by the active or the placebo from a DBPCFC (159, 160). The procedures around DBPCFC have been standardized regarding patient selections, settings and challenge procedures but there is still lack of information and consensus on how to optimize the recipes used for blinding and also if the nutritional content of the challenge plays a role for the outcome (161). The recipes used for blinding food must be adjusted with regard to the patient and the suspected allergen, In general, the recommendations are to use as few hypoallergenic ingredients as possible when hiding an active ingredient (162). When performing DBPCFC for peanut it is particularly important to hide the peanut taste and texture in order to ensure that the challenge is really blind (163).

1.9.5 Basophil activation test (BAT)

Blood basophils are granulocytes that develop from CD34+ pluripotent progenitor stem cells, differentiate and mature in the bone marrow and then circulate in the peripheral blood. Basophils typically comprise <1% of the circulating leucocytes. Basophils are functionally similar to mast cells, and can be activated by a number of stimuli which are mediated by the high-affinity IgE receptor (FcεRI) and are able to secrete inflammatory mediators and cytokines upon stimulation. Early studies of the role of basophils in allergic disease focused on mediator release, such as histamine and leukotrienes (164). Histamine is one of the four types of mediators which is released immediately by the basophils. Phospholipid metabolites, such as leukotriene C4 (LTC_4) and IL-4 as well as other cytokines, represent newly synthesized mediators which are released more slowly Lastly, it is possible to induce basophils to synthesize a mediator (which may take 18-24 hours) that is stored in the existing granules for subsequent immediate release. Besides the mediators' release, basophils also increase activation markers on their surface after degranulation. It is possible to perform assays on the mediator release in whole blood but the methods required to measure the histamine release are onerous and detection of other mediators often require basophil enrichment and cell culture along with auto-analyzers for released histamine or ELISAs for LTC_4 and IL4.

The development of a flow cytometry-based functional basophil activation assay became possible in 1991, when Knol et al. demonstrated that a surface marker, CD63, is upregulated on basophils at the same time as basophils degranulate (165, 166). This means that CD63 is up-regulated on the surface of basophils by cross-linking of the high affinity IgE receptors (FcεRI) with the allergen. With this method it is possible to stimulate the cells with the allergen in heparinized whole blood and then analyze their degree of activation (167). Surface expression of $CD203_c$ is used to identify the basophils in the peripheral blood and the up-regulation of CD63 on their surface is used to identify if they are activated. Several other activation markers have also been identified on basophils, such as CD69, CD11, CD13, CD107a, CD107b, CD164, but their clinical use has not yet been established (167).

Basophil degranulation

Two pathways of basophil degranulation have been demonstrated with the help of electron microscopy; the "piecemeal and the "anaphylactic" degranulation. In anaphylactic degranulation the intracellular granules empty their whole content rapidly by extrusion via exocytosis. In piecemeal degranulation though, the cells secrete parts of the granule content without exocytosis (168).

Basophils express unique surface markers depending upon whether cells undergo anaphylactic or piecemeal degranulation, which can be measured by flow cytometry. The surface markers that best indicate anaphylactic degranulation is CD63.

CD63, also known as lysosomal-associated membrane glycoprotein-3 (LAMP-3), is a 53-kDa member of the transmembrane-superfamily (tetraspanins). In a resting basophil, this protein is located on the membrane of intracellular secretory granules. After stimulation by FcεRI, these granules fuse with the plasma membrane and thus, CD63 is expressed on the surface of the degranulated basophils. Early studies suggest that CD63 up-regulation can be used as an indirect marker of histamine release (169). However, recent studies suggest that histamine release is the sum of both anaphylactic and piecemeal degranulation, and up-regulation of CD63 may be representative of only anaphylactic degranulation (170). **CD203c**, a glycosylated type II transmembrane molecule that belongs to the family of etonucleotide pyrophosphatase/phospshodiesterase enzymes and is present on $CD34^+$ progenitor cells, basophils and mast cells. CD203c is constitutively expressed at low levels on the basophils surface membrane and is quickly up regulated upon cell activation via allergen, but more slowly in the presence of IL-3. This up-regulation differs from CD63 in terms of the inciting stimuli and early signaling events, which suggests that CD203c may be associated with piecemeal degranulation (166).

Technical Considerations of the Basophil Activation Test

When performing the basophil activation test some technical aspects should be considered when interpreting the results. Correct sampling and preservation of the blood is of critical importance to obtain optimal cellular viability and functionality especially with basophils. Ideally, analysis should be performed within 4h after sampling (heparin or EDTA tubes), kept in room temperature. Analyses could be performed not later that 24h, since it was shown that after this, the basophil lose their reactivity by 22-48%, depending on the anti-IgE dilution, After 48h the basophils responses are reduced by 42-58.5%, but even after 4h a slight reduction at usual dilutions of anti-IgE has been observed (171), therefore BAT should preferably be performed using freshly drawn blood. To control for the decay of the basophil responsiveness a positive control stimulus with an activating anti-IgE or anti- FcεRI is normally included in the assay (see below).

Correct interpretation of the BAT needs relevant negative and positive control stimulation to be included. The negative control stimulation assess

spontaneous expression of the activation markers on the basophils. In the positive control the basophils are stimulated with a cross-linking antibody to IgE or the FcεRI sometimes together with general stimulator e.g. fMLP, that normally fully activates all the basophils. In the absence of positive control stimulation, it becomes impossible to correctly interpret negative results of allergen challenges and even then approximately 5-10% of the individuals tested fail to upregulate CD63 and/or $CD203_c$ after positive control stimulation. For these "non-responders" the BAT is lost as a diagnostic instrument, whereas for study purposes, patients with unresponsive basophils should be recorded as false negatives. False-negative results might also be explained by other causes such as recent exposure to the allergen with refractoriness of the cells and/or transiently reduced levels of allergen-specific circulating and membrane-bound IgE. Negative BAT results can also have technical causes such as improper handling, storage, inappropriate challenge of the cells with poorly identified allergens that contain cytotoxic or inhibitory components, application of cytotoxic stimulation concentrations (172).

Some authors have shown that a short pre-incubation with IL-3 might increase the sensitivity of CD63-based assays but this seems not to be crucial for proteinaceous allergens and stimulation with serum of patients with autoimmune urticarial disease (173, 174). Flow-assisted analysis of in vitro-activated basophils can be applied either on whole blood or isolated cells. The advantage of using whole blood is that it needs less manipulation, and the presence of all blood components might better mirror the physiological or pathophysiological in vivo conditions. Separation of basophils is accompanied by loss of basophils, and *in vitro* activation of the cells might occur. Pre-warming of cells has no effect on the kinetics of signaling molecules when fresh blood is used. Proper selection of the allergen for stimulation is needed and it should be kept in mind that allergens used for stimulation may not be homogenous, and may have varying compositions, or contain components with nonspecific stimulatory/inhibiting effect such as preservatives, endotoxins and lectins. To some extent, applying isolated or recombinant allergens, if available, can circumvent these issues and make the assay more reliable (175-177).

Treatment with antihistamines do not interfere with basophil reactivity as they block only the effect of histamine and not the mediator release from basophils or mast cells. Glucocorticoids reduced basophil reactivity only at unusually high concentrations in vitro with a short incubation time (171).

Clinical applications of the Basophil Activation Test

The basophil activation test (BAT) has in recent years been applied for the diagnosis of allergy and provides a new promising diagnostic tool (178). BAT has been used for the diagnosis of drug allergy such as antibiotics (179-181), NSAID (182-184), radio contrast media (185), anesthetics (186, 187), and also for latex (177) with various results. BAT was also applied for the diagnosis of inhalation allergens such as grass pollen (188, 189) and house dust mite (190) as well as for the diagnosis of allergy to insect venom (191-194). In addition BAT has been used for monitoring the treatment effect of immunotherapy for grass and insect venom allergy (195, 196).

BAT was used for the first time by Moneret-Vautrin and colleagues in 1999 for the diagnosis of food allergy to 10 different foods in allergic patients (178). BAT was later applied in the pediatric population for the diagnosis of allergy to hen's egg and cow's milk (197, 198), and for the diagnosis of IgE-mediated wheat allergy (199). BAT has also been tested in the adult population for the diagnosis of pollen-associated food allergy (175, 200) as well as in single cases as for the diagnosis of beef-induced anaphylaxis (201) and in the diagnosis of allergy to sesame with co-existent sensitization to walnut (202). Interestingly BAT was also applied in adult patients with IBS-like symptoms for the diagnosis of food hypersensitivity to milk and wheat (203). In addition BAT has been used as a predictive diagnostic tool before an oral challenge with cow's milk (204) as well as for the monitoring of specific oral tolerance to egg in children (205). In the field of peanut allergy, BAT has been used as a complementary tool for the evaluation of peanut allerginicity after thermal procession (206). In peanut allergic children a good correlation could be observed between the BAT and the outcome of an accompanied oral food challenges (207), and in a recently published study BAT was shown to be a useful diagnostic tool in order to discriminate peanut allergic from peanut sensitized children (208).

1.9.6 Diagnostic approach in patients with gastrointestinal food allergy

In patients with food allergy, symptoms vary from marginal impairments to life-threatening shock reactions. Major targets of food allergy are the skin, the respiratory tract and the gastrointestinal tract, either alone or in combination. Gastrointestinal symptoms like nausea, vomiting, abdominal pain and diarrhea appear typically in combination with allergic manifestations in the skin and

other target organs. The airways are often not considered as a common site of involvement, but about 10–20% of patients with gastrointestinal hypersensitivity disorders show involvement of the respiratory tract (209). Objectively confirmed food allergies occur in 2–8% of the pediatric population (children from 2–3 years are the most affected group) and in 1–3% of adult persons (highest prevalence between 20 and 40 years). Food allergy is reported by 25–50% of individuals with gastrointestinal disorders (210, 211). A study (212) of 164 children (from 6 months to 18 years) suffering from atopic dermatitis (with associated food allergy in 80% of the patients) reported gastrointestinal disorders in approximately 60% of the cases. Eighty per cent of patients suffering from eosinophilic gastrointestinal disorders showed co-existing atopy, whereas anaphylaxis to food co-exists in approximately 20% of these patients (213). Approximately 80% of children with cow's milk allergy develop clinical tolerance when they reach five years of age, whereas 35% develop other food allergies (214). The diagnosis of gastrointestinal-related food allergy is based to a large extent on exclusion of other gastrointestinal diseases such as carbohydrate intolerances, histamine intolerance, celiac disease, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), gastro-esophageal reflux disease, chronic infectious gastrointestinal disease, malignant gastrointestinal diseases, extra-intestinal inflammation and mastocytosis (16).

The evaluation of suspected GI food allergy begins with a careful history of symptoms that correlates to specific foods together with SPT and/or IgE-tests. Most immediate hypersensitivity reactions to food include a set of symptoms that consistently occur minutes to hours after ingesting certain foods. In some individuals, other factors such as medications or exercise may modulate the reaction to a specific food. Specificity of the reaction does not always imply a food allergy because even patients with reactions from a non-immunological mechanism, such as lactose intolerance, may report defined reactions to specific foods. If a specific food or group of foods cannot be identified by the initial history, the patient should keep a diet diary for several weeks in an attempt to correlate foods with GI and other symptoms. After that certain foods are identified as possible culprits, and these items should be eliminated from the diet for several weeks to determine the effect on symptoms. If a benefit is seen, the patient may reintroduce the putative allergen(s) in an attempt to prove the association. Such open food challenges are subject to bias and should be corroborated by another more objective method, usually DBPCFC, before permanent elimination of the offending food item from the diet. This is of particular importance if the patient is young and the foods in question represent a major component of the diet such as eggs, milk, or wheat. If specific foods are not identified by the clinical history or by a diet diary, a hypoallergenic diet may be tried for about 3 weeks. In cases where a benefit is seen, new foods are

gradually introduced in an attempt to identify specific foods that may contribute to the illness (210).

In case of suspicious local allergic gastrointestinal enteropathy, when the diagnosis of food allergy cannot be set by the conventional allergy investigation, another possible diagnostic approach is endoscopy and collection of a biopsy from the intestinal mucosa. The histological pattern of the mucosal pathology is of importance for the exclusion of other non-allergic disease in the gastrointestinal tract as well as for the choice of right treatment- or further investigational strategy in case of suspicious local food allergy in the intestine.

Other experimental approaches have been described such as a local challenge of the intestinal mucosa with the suspected allergen (215). Capsule endoscopy is not used routinely for investigating suspicious food intolerance but an interesting study by Hagel et al showed that small-bowel capsule endoscopy of food allergic patients revealed that 93% of patients had signs of non-erosive lesions such as erythema and swelling in the small bowel (216). Even cases with supposedly non-IgE mediated local food-induced hypersensitivity in the gut were explored with ultrasonography and magnetic resonance imaging after challenge with the offending food with results suggesting that these two diagnostic tools may be of value for documenting such responses (79). The use of mucosal patch test in the colon (rectal protein challenge) has been tested for the diagnosis of food intolerances, mainly cow's milk and gluten, and in patients with IBS as well as patients with autoimmune manifestations. The test reveals locally increased concentrations of nitric oxide, myeloperoxidase neutrophilic or eosinophilic granule constituents (217-219). Finally, when suspecting intestinal mastocytosis the investigation may be more complex since this requires intestinal biopsies from multiple sites of the GI tract (stomach, duodenum, ileum, colon) and the assessment of biopsies by an experienced pathologist in order to set this diagnosis (220, 221). An algorithm for the investigation of gastrointestinal food allergy is presented in Figure 5.

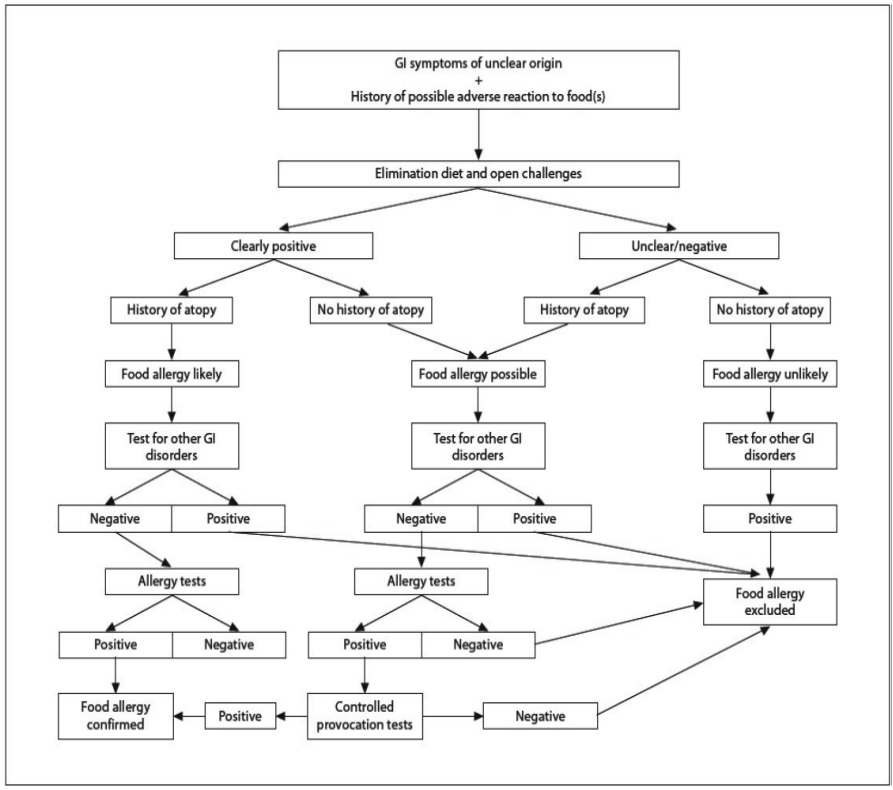


Figure 5. The diagnostic food allergy flowchart, adapted by Bischoff and Crowe (210).

1.10 Clinical implications of birch pollen and birch pollen associated foods

Birch pollen is one of the most common causes of rhinoconjunctivitis and allergic asthma in Northern and Central Europe and North America. It has long been known that patients with birch pollen allergy may develop immediate reactions to fruits and vegetables in addition to seasonal respiratory symptoms. This birch-fruit-vegetable syndrome is characterized by local symptoms at the site of food contact such as itching of the lips, tongue, and throat, sometimes accompanied by swelling of the lips and tongue, referred to as the oral allergy syndrome (OAS) (5). Occasionally, severe systemic IgE-mediated reactions

such as urticaria, asthma, or anaphylactic shock may occur. Although birch pollen–related food allergy is often noticed in clinical practice, no recent data on the prevalence and main triggers of this type of food allergy are available. Birch pollen–related food allergy may be a consequence of immunologic cross-reactivity between ubiquitous birch pollen allergens and structurally related food proteins. IgE antibodies specific for the major birch pollen allergen, Bet v 1, have been shown to cross-react with homologous proteins identified in different stone-fruits, such as apple (Mal d 1), cherry (Pru av 1), and pear (Pyr c 1), as well as hazelnut (Cor a 1), celery (Api g 1), carrot (Dau c 1), soybean (Gly m 4), peanut (Ara h 8), jackfruit and kiwi (Act d 8) (222). These food allergens can also activate Bet v 1–specific T cells to proliferate and produce cytokines, sometimes even resulting in T cell–mediated late-phase responses, such as flare-ups of atopic eczema in the skin (223, 224). Bet v 2, the birch pollen profilin, is another allergen able to induce cross-reactive IgE antibodies (225). Bet v 2–specific IgE antibodies have been shown to recognize profilins in apple, banana, carrot, celery, cherry, hazelnut, pear, pineapple, potato, and tomato (222). Profilin sensitivity rarely gives clinical reactions in patients due to cross-reactivity but a recent study by Alavarado et al. showed that IgE to profilins may be related to severe allergic reactions (226). In addition IgE antibodies specific for the minor allergen Bet v 6, have been shown to cross-react with proteins of comparable size in apple, banana, carrot, lychee, mango, orange, and pear (227).

Often patients with birch pollen allergy, who display IgE reactivity to birch pollen–related allergens in foods, do not develop clinical symptoms when consuming the foods, and there are no published data available concerning the exact prevalence of OAS after intake of birch related food. There is evidence that patients with birch-pollen allergy may experience gastrointestinal symptoms during the birch pollen season which may suggest that these symptoms are caused by the ingestion of birch-pollen related food (67, 68). This is supported by the finding that some patients recover clinically from their OAS or experience milder symptoms when ingesting birch pollen-related foods after immunotherapy for birch pollen, but currently, there are very few studies and case reports available supporting this clinical observation (11, 228-232).

1.11 Food allergy and asthma

Food hypersensitivity or allergy to foods in adult patients with asthma is very little explored until now. Most of the data concerning the relationship between food intolerance and asthma are coming from studies and surveys done in the pediatric populations. Similar data from the adult population are only available from a few minor studies and case-reports (233). Food allergy reactions can involve the respiratory tracts with symptoms from asthma and stridor besides the skin and gastrointestinal tract (112). Wheezing is a common manifestation of food allergy in association with other systemic symptoms (234), and an increase in the prevalence of asthma and allergy has been noticed since about 1960. Between 1960 and 1980 the incidence of self-reported asthma in the United States was increased from 2.5/1000 to 6.0/1000, and it was estimated that an annual average of 20 million people obtained the diagnosis of asthma between 2001 and 2003. Of these 6.2 million were children less than 18 years-old (235). The prevalence of food allergy has also increased in the past 10 to 15 years and in the USA varies between 2-5% and it is greater in the pediatric population than in adults with estimates of 6-8% in children under 5 years old and 3-4% in adults (236, 237). Reports from Europe estimate the prevalence of food allergy to be between 0.3 and 7.5% in children and 2-4% in adults (238, 239). Food allergy has also been observed more frequently in individuals with atopic dermatitis (240). Co-existent food allergy and asthma, account approximately for one third of children with food allergy, and about 4-8% of children with asthma have food allergies (241, 242).

The most common foods causing reactions from the lower respiratory tract, with wheezing as the most common symptom, after ingestion or exposure to aerosolized particles of the allergen are fish, egg, shellfish, peanuts and tree nuts. Peanuts and other tree nuts have been implicated as causing allergic reactions when aerosolized in an airplane and the most widespread epidemic of airborne food-induced asthma has been reported in Barcelona after exposure of airborne soy dust (243, 244). Different studies performing blinded challenges in children with food allergy confirmed that respiratory reactions are most often caused by peanut, tree nuts, egg, milk, soy, fish and shellfish (245-248). In a study on adults by Woods et al it was shown that peanut and shrimp allergics have more frequent asthma episodes and doctor's diagnosed asthma (249). There is little evidence supporting a possible relation between food additives and asthma but from the very few studies available, it was concluded that sulfites may induce asthma with a prevalence of less than 3.9%, and intolerance to sulfites was found to be more prevalent in those with steroid-dependent asthma (250). In adults, it has been shown that aerosolized food particles may lead to the development of asthma. A typical case of

occupational asthma is Baker's asthma which is estimated at about 0.3% per year (250). Other foods which are implicated to cause occupational asthma include egg, shellfish, enzymes used in the cheese industry, milk and carob bean flour (251-255). In addition, food allergy has been identified as an independent risk factor for asthma morbidity with higher fatality rates in children with peanut or other food allergies in combination with asthma (256-258).

A relationship between asthma and concomitant food allergy has also been observed in adults where patients with more than one food allergy had increased hospitalizations for asthma (259). This finding is in the line with another study from Sweden in which it was shown that multiple IgE-sensitization to foods, increase the frequency of co-existent asthma in adults (260). An interesting and unexplored issue is the relationship of asthma and gastrointestinal symptoms in patients with food intolerances. Caffarelli et al., showed that children with asthma reported more gastrointestinal symptoms compared to controls and that gastrointestinal symptoms were more frequent in children with other atopic manifestation than asthma or which were SPT-sensitized to foods (261). Powel et al. confirmed these findings in the adult population, where patients with asthma generally experienced more gastrointestinal symptoms than the non-asthmatic population (262, 263). There is, however, very limited data on the prevalence of food hypersensitivity or allergy to common foods in adults with asthma, and which foods that most often cause gastrointestinal symptoms in adult asthmatics. Previously though, it has been clearly shown that there is a relation between the hyper-reactivity in the lower airways and an increased inflammatory activity along with increased intestinal permeability. Patients with IBS and IBD were shown to have increased bronchial hyper-reactivity, when comparing the FEV1% and positive metacholine test, than control subjects (264, 265). Similar results were observed when comparing patients who suffered from asthma with asymptomatic atopic subjects (266). Finally, an increase of the intestinal permeability in patients with asthma (83) as well as in patients with atopy and IBS (66) compared to non-atopic subjects has also been shown.

1.12 Food allergy and psychological impact

It has been reported that food is involved in numerous psychological and somatic disorders with psychological overtones such as anorexia, bulimia, obesity, panic, depression and many others. Food-related behavior has not been the means of expression of psychological disorder, but food itself has been

implicated in the causation and exacerbation of emotional and psychological problems, however allergic patients do not generally have more psychiatric disorders than non-allergic patients (267). Food allergy may cause additional psychological burden of dietary restriction and vigilance and continuous anxiety regarding the consequences of accidental exposure (48). Peanut-allergic children, as reported by their parents, were found to have significantly more disruption in their daily activities and increased impairment of familial social interactions compared to the families of children with a rheumatological disease. The opposite was true for peanut-allergic adults who scored worse on mastery and coping mechanism associated with their disease, but had less personal strain and familial disruption than adults with rheumatological disease. This difference was not only attributed to greater vigilance that the parents practice over the management of their children's allergies, leading the better mastery and coping, but also to a higher stress levels. Peanut allergic adults were less compulsive regarding management of their own allergies, and thus had less stress and social disruption (268). This study also emphasized the significant psychological burden of a food allergy diagnosis on families and their need for educational and emotional support. Other studies found that food allergy had significant effect on meal preparation, family social activities, stress levels and school attendance (269). Peanut-allergic children were found to have poorer quality of life and had more fear of adverse events, anxiety about eating, restrictions in physical activities compared to children with insulin-dependent diabetes, and felt safer when they ate in familiar places or when carrying epinephrine kits (270). Adolescents and young adults are especially vulnerable and have been found to be particularly in higher risk for fatal reactions while findings from other studies in this group of patients revealed risk-taking behavior such as "ingesting potentially unsafe food" and failure to "always" carry epinephrine which lead to increased sense of "social isolation" or "feeling different".

Many recent reports imply a possible relation between food allergy and psychological disorders. A British group of physicians in the beginning of the 80s defined two main disorders: food intolerance or adverse reaction to a specific food that may be verified under-blinded challenge conditions; and food aversion or "pseudo-food allergy" which includes psychological avoidance of food and psychogenic reaction to food due to emotions associated with the food rather than a physical response to the food itself (48). Food aversion may be accompanied by neophobia in some individuals, meaning that an individual with food aversion is becoming suspicious for testing new foods, which will have consequences for the individuals diet habits and behavior (63). Patients with pseudo-food allergy can be identified as having adverse reactions to specific foods, but in the absence of objectively recognized signs and symptoms, physical findings, and laboratory evaluation supportive of an

allergic, toxic, enzymatic or pharmacological reaction to a specific food. These patients will not reproduce objective symptoms or physical changes under adequately performed DBPCFC. It has been shown that food intolerance has a significant impact on the aggravation of symptoms in patients with IBS (65) and that patients with IBS have an increased sensitivity to different stimuli such as food intake and distension. This syndrome it is believed to be caused by increased visceral perception (chronic visceral hyperalgesia/hypersensitivity). Therefore, different psychotherapeutic methods e.g. hypnosis has been proposed in IBS therapy (63). Previously, it has been reported a relation between autism and food allergy, where gluten and milk sensitivity has been proposed to play role (271). This was supported by the increased basophil degranulation in response to food allergens observed in 10 autistic children (272). In addition, it has been reported that elimination of cow's milk from the diet resulted in improvement of behavioral disturbance in children with autism. Supportive of an immune mediated inflammation of the GI tract is the presence of lymphocytic infiltration in the upper GI-tract, immune activation and an abnormal lymphocytic response to dietary antigens seen in children with autism (273, 274). Aggravation of the symptoms of schizophrenia by gluten intake have previously been reported and elimination of gluten could therefore have a therapeutic value in these patients (275). However, no increased incidence of celiac disease (CD) was found in patients with schizophrenia (275, 276). On the other hand, high prevalence of anxiety, depression, and disruptive behavioral disorders has been reported in adults and adolescents with CD (277, 278), which were significantly improved after 3 months of gluten-free diet (279). Finally, food intolerances/sensitivities have been reported to be frequently associated with idiopathic environmental intolerance or so called multiple chemical sensitivities (MCS) (280, 281).

1.13 Treatment of food allergies – where are we now in 2015?

Currently there are no curative treatments for food allergy or effective means of preventing disease other than avoidance. The current guidelines for the management of food allergy include strict dietary avoidance, modified diets depending of the diagnosis (e.g. diet free form biogenic amines, six-food-diet, FOODMAP, hypoallergenic diet etc.), nutritional counseling and emergency treatment in the setting of accidental ingestions (10, 282). Considerable effort has gone into the development of strategies aimed in curing food allergies over the past 15-20 years. Fortunately, there are a number of therapeutic strategies currently being investigated for the treatment and prevention of food allergy.

There are both allergen-specific and nonspecific approaches. Allergen-specific approaches have largely focused on administering gradually increased doses of antigen via various routes, either subcutaneous (peanut), oral immunotherapy (OIT) (peanut, cow's milk, egg, fish and fruit), sublingual immunotherapy (SLIT) (peanut) or epicutaneous immunotherapy (EPIT) (cow's milk) (283, 284). To date, food-specific immunotherapy have been successful at achieving desensitization, however evidence of sustained tolerance has not been shown yet. Recently, the addition of Anti-IgE mAbs to immunotherapy regimens has also been explored as a potential means of improved safety and a shortened time to achievement of maintenance dosing (peanut).

Non-specific approaches include the use of Chinese herbal formulation (FAHF-2), which prevented peanut-induced anaphylaxis in a murine model (285). The use of anti-IgE mAbs to reduce the threshold dose to reactivity to various food allergens (peanut) is also being investigated (286). The use of probiotics has also showed an improvement in the intestinal microbial balance which may lead enhanced immunological maturation and to a more tolerogenic environment. The major sources of probiotics are dairy products that contain Lactobacillus and Bifidobacterium species. Potential mechanisms of probiotic immunomodulation include increased synthesis of IgA and IL-10, suppression of TNF-α, inhibition of casein-induced T-cell activation and circulating soluble CD4+, and toll-like receptor 4 signaling (287). Clinical trials have focused on the prevention and treatment of atopic dermatitis in children with food allergy (288). Prenatal supplementation of mothers with probiotics showed a decrease in the prevalence of atopic dermatitis (289, 290). In mouse-models the administration of probiotics prior to allergen sensitization diminished anaphylaxis severity and increased specific IgA response in the gut (291). In a mouse model of shrimp anaphylaxis, oral administration of a mixture of probiotics reduced the symptom scores (292). Parasitic helminth infections have shown to ameliorate the allergic response in a murine model of peanut allergy with decreased production of peanut-specific IgE, therefore the use of Trichuris suis eggs in humans with food allergy is also being investigated (293).

These various therapeutic strategies represent just a part of the broad array of investigations in search of a treatment of food allergy, but these therapies are not yet clinically available (287).

2 AIM

The aim of this thesis was to explore the pattern of gastrointestinal inflammation in birch pollen allergic patients and to evaluate, the use of basophil activation test in food allergy with focus to peanut allergy and to examine the relation between food hypersensitivity and asthma in the adult population.

Specific aims:

Study I:

The aim of this study was to explore the immune pathology of the duodenal mucosa in birch pollen allergic patients, with or without GI symptoms, outside and during the birch pollen season. In addition, we aimed to relate these findings to the IgE antibody profile against birch related food allergen components in these patients. The results contributed to the understanding of the etiology of GI symptoms in pollen allergic patients.

Study II:

The aim of the presents study was to investigate whether patients who have suffered a severe allergic reaction to peanuts or who have been designated as being allergic to peanuts since childhood, can be diagnosed with clinical or persistent peanut allergy using the BAT. We also examined whether the BAT could discriminate between patients with severe peanut allergy and sensitized patients with no or mild symptoms in order to evaluate if a person is no longer severely allergic to peanut even when displaying persistent IgE-mediated peanut sensitivity, as assessed using conventional tests, including reactivities to allergen components. In addition, we asked if the BAT can be used for the diagnosis of co-existent concomitant allergy to soy in patients who are sensitized or allergic to peanuts, and if any possible underlying clinical cross-reactivity among legumes can be revealed by combining BAT reactivity and IgE sensitization profiling to allergen components.

Study III:

The aim of this study was to explore the prevalence of self-reported adverse reactions to foods and to estimate the prevalence of IgE sensitization for the most common foods among adults with asthma compared to non-asthmatics. We also wanted to determine the spectrum and the prevalence of gastrointestinal symptoms caused by the most common foods in both asthmatics and non-asthmatics.

3 PATIENTS AND METHODS

STUDY POPULATION

Study I:

Patients and healthy controls were recruited from the Asthma and Allergy clinic at the Sahlgrenska University Hospital in Gothenburg, Sweden. The patients were requested to answer a detailed questionnaire about their birch pollen related symptoms and if and when they had gastrointestinal symptoms (supplement attached in the Original Study I). All patients included were between 18-50 years old and allergic to birch pollen. Some of the patients were also allergic to other allergens, and some were diagnosed with oral allergy syndrome (OAS) and/or asthma (Table 2). The schematic flow for the selection of patients and healthy volunteers is presented in Figure 6. Exclusion criteria for all subjects were confirmed inflammatory bowel disease, celiac disease (none of the subjects were positive for transglutaminase or gliadin antibodies in serum) or other gastroenterological disease, food allergy to staple foods (egg-white, milk, wheat, soy, peanut, codfish), pregnancy, lactation, rheumatic or systemic disease, immune deficiency, previous or current treatment with immunotherapy. The gastroscopies were performed only as part of this study. All gastroscopies were executed by experienced gastroenterologists, and no signs of macroscopic pathology or abnormality were found neither in the esophageal nor in the gastric or duodenal mucosa in any of the patients.

The birch and grass pollen counts (pollen grains/m^3) during 2007-2010 in the area where the patients were recruited are presented in the supplementary Figure in the Original Study I.

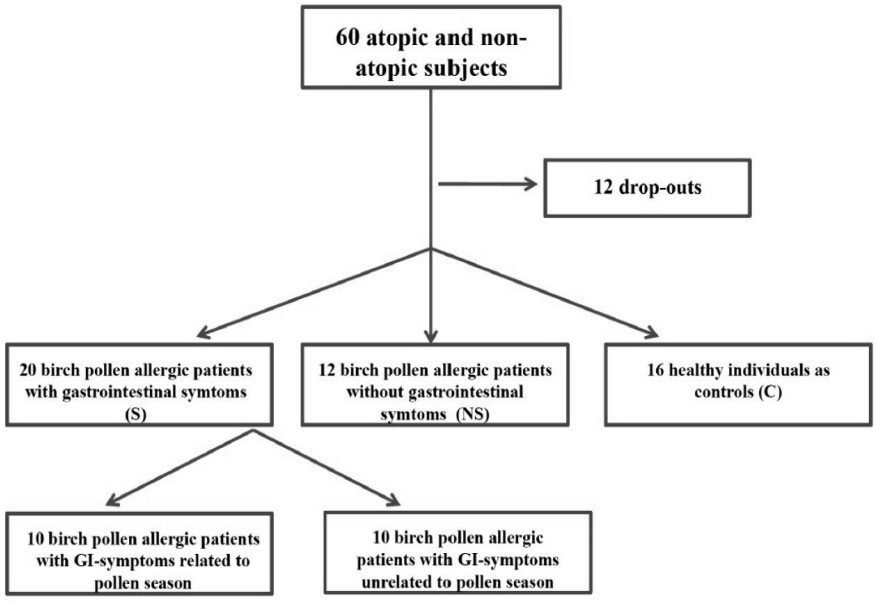


Figure 6. Overview of the selected study populations.

Study II:

Forty-seven adults with severe allergy to peanuts (PA-group), 22 peanut-sensitized persons (PS-group) and 22 healthy controls (C-group), all in the age range of 18–60 years, were recruited either retrospectively or prospectively to the study between January and December of 2013. All the patients had been referred to the Allergy Clinic at the Sahlgrenska University Hospital in Gothenburg for allergy investigation. The PA-group consisted of patients with severe peanut allergy who had a convincing history of anaphylaxis to peanuts with objective symptoms, together with the routine allergological investigation including a detailed clinical history and/or IgE titers to rAra h 2 >0.35 kU/L. The PS-group consisted of patients who had previously undergone investigations with oral peanut challenge for suspected peanut allergy due to a co-existing allergy to birch pollen. The majority of the patients in the PA-group and PS-group were also sensitized to soy and/or birch pollen. There were no drop-outs during the study. All patients and controls were investigated using the SPT for peanut, soy and birch, as well as measurements of total IgE and

specific IgE for peanut, soy, and birch. Blood was collected from each subject for the BAT and serum was saved for analysis of sensitization to allergen components (ISAC). Exclusion criteria for all the subjects were: pregnancy; lactation; rheumatic or systemic disease; and immune deficiency. Five patients in the PA-group and three in the PS-group had previous or currently ongoing treatment with immunotherapy for pollen allergy (birch and grass allergy). All the patients in the PS-group answered a questionnaire regarding whether they had eaten peanuts after they had undergone a negative open oral challenge with peanut.

Study III:

Study area

Study III was part of the larger study West Sweden Asthma Study (WSAS) that took place in 2008 and it was completed in 2012. Subjects from the region Västra Götaland, which reaches from the northern part of Sweden's west coast to the central part of Southern Sweden were included. The region is very diverse with rural areas small and medium sized towns and a big city. In the beginning of 2008, when the study was initiated, 1.6 million people were living in this region. Gothenburg is situated on the west coast and is the second largest city in Sweden with 700 000 living in the city or in the surrounding urbanized area. The population in this area of Sweden is representative, in regards to age and gender distribution. The climate is oceanic according to Köppen climate classification with warm summers, mild winters and high humidity. The average temperature is between 15 and 16 ºC in July and between -1 and -4ºC. The average precipitation is 500-1000 mm/year (294).

Postal questionnaire

A postal questionnaire was mailed out to randomly selected subjects. The questionnaire consisted of two parts that were included in a folder mailed to selected subjects, together with a pre response envelope. The participants could also choose to respond by using a web based questionnaire with unique user names and passwords. The first part of the questionnaire was Obstructive Lung disease in Northern Sweden (OLIN)-questionnaire (295), which was extensively used in Sweden and within the FinEsS-studies (epidemiological studies in Finland, Estonia and Sweden) and has also been used in Vietnam. The second part consisted of the Swedish version of the GA^{2}LEN-questionnaire (296). The two questionnaires complement each other as the

OLIN-questionnaire more thoroughly covers bronchitis and chronic obstructive pulmonary disease (COPD), while the GA^{2}LEN-questionnaire has detailed questions on rhinitis, chronic rhinosinusitis and eczema. From the questionnaire, the definition of multi-symptom asthma (MSA) was created. This definition includes subjects who report physician-diagnosed asthma and asthma medication and attacks of shortness of breath and recurrent wheeze and at least one respiratory symptom (294).

Study population

The above postal questionnaire, which has been described also in detail elsewhere (297), was mailed out to 30000 randomly selected subjects, aged 16-75 years, living in the West of Sweden; 15000 subjects lived in the urban area of Gothenburg and 15000 in the remaining region of West Sweden. The total response rate was 62 %. A non-response study showed no differences in prevalence of asthma symptoms or lung disease between responders and non-responders (297). Of the responders to the postal questionnaire, 2000 were randomly selected for clinical examination and interviews. In addition, all responders that reported physician diagnosed asthma, or reported ever having asthma and used asthma medication or reported symptoms such as wheeze or attacks of shortness of breath during the last year, were included. In total, 3524 subjects were invited, of which 2006 participated. All participants received a questionnaire containing detailed questions on food hypersensitivity as well as other hypersensitivity symptoms (Appendix 1: Questionnaire in the Original Study III). The questionnaire did not contain specific questions on gluten (coeliac disease) or lactose intolerance. Of the 2006 participants, 1725 responded to the food questionnaire of which 1527 were included in the analyses, 583 of these were diagnosed as asthmatics.

The clinical assessment of the subjects in the study included spirometry, blood samples for specific IgE-tests and a clinical interview performed by a specialist nurse. The clinical interview was used to assess whether the subjects currently suffered from asthma. This was defined as: a) asthma diagnosed by physician, and reported asthma symptoms or asthma medication during the last year, *b)* belief to have suffered from asthma, and currently report asthma symptoms and/or taking asthma medication, *c)* currently suffer from asthma symptoms and have either positive methacholine bronchial challenge test or positive reversibility test.

A schematic flow chart of the study set up can is presented in Figure 7.

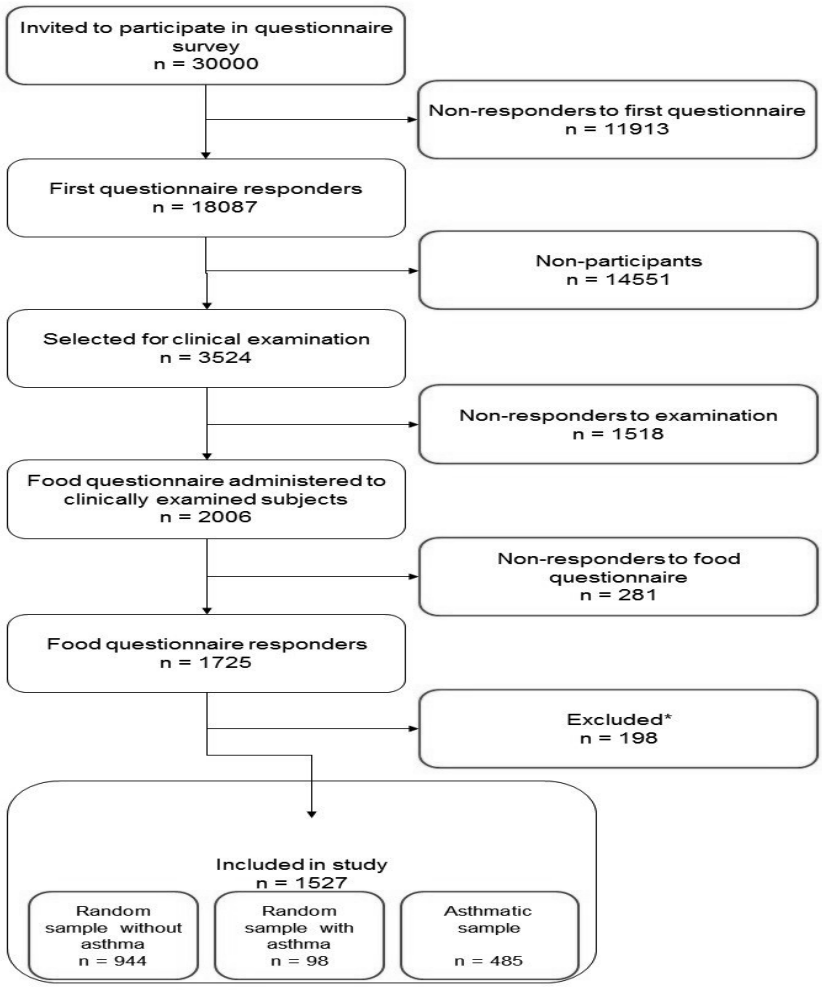


Figure 7. Number of selection, responders and non-responders. * 198 subjects were excluded from the study since their initial categorization as asthmatics, based on the questionnaire response, were considered inappropriate after clinical examination.

METHODS

Allergy and IgE-sensitization assessment

Skin prick test (SPT)

Skin prick test was performed, by using allergen extracts from birch, timothy, mugwort, animal dander (dog, cat, horse), dust mites, peanut and soy (Soluprick, ALK-Abelló, Hørsholm, Denmark). Histamine (10mg/ml) and vehicle was used as reference. Skin prick test was considered positive when the wheal reaction diameter was 3 mm or more.

ImmunoCAP

In *study I* the serum levels of total IgE and IgE antibodies to pollen allergens (*birch, grass, mugwort*), and a mix of common food allergens (fx5; *egg-white, milk, wheat, soy bean, peanut, codfish*) were measured using ImmunoCAP (Thermofischer Scientific, Uppsala, Sweden) according to the manufacturer's instructions. In *study II*, the serum levels of total IgE and the serum levels of birch, peanut and soybean were measured using ImmunoCap. In *study III*, specific IgE-tests included three allergen panel tests, Phadiatop Europe (*cat, dog, horse, Dermatophagoides pteronyssinus, Dermatophagoides farinae Cladosporium herbarum, timothy grass, birch, mugwort, olive, wall pellitory*), fx1 (*peanut, hazel nut, brazil nut, almond, coconut*) and fx5 (*egg white, milk, fish, wheat, peanut, soy bean*) (Thermofisher Scientific, Uppsala, Sweden). Subjects with a positive response to any of the above panels were additionally tested specifically for the IgE of the allergens included in this positive panel, according to manufacturer's instructions.

ISAC

For *study I,* blood samples were collected during the birch pollen season (5th May until 10th June) and outside the birch pollen season (1st October until 5th March), between 2008-2010, for the determination of IgE against allergen components using the allergen microarray immunoassay, ImmunoCAP ISAC® (Thermofischer Scientific, Uppsala, Sweden) on which 103 allergen components are spotted.

For *study II,* the determination of IgE against allergen components were measured by using the latest version of micro-array Immunoassay ImmunoCAP ISAC® (Thermofischer Scientific, Uppsala, Sweden) that covers

112 components from 51 allergen sources. The results are expressed as ISAC Standardized Units (ISU) with a threshold of >0.3 ISU. The ISAC analyses was performed according to the manufacturer's recommendations.

Gastroscopy and duodenal biopsies

Gastroscopies and duodenal biopsy sampling were performed by gastroenterologist at the Department of Endoscopy in the gastroenterology clinic, Sahlgrenska University Hospital during the pollen season between the 5th of May until 5th of June, and outside the pollen season between 1st November and 5th of March. Biopsy specimens (6-7 specimens 2 to 3 mm in size) from the descending part of the duodenum were obtained. The tissue specimens were immediately embedded in O.C.T. (Optimal Cutting Temperature) compound, frozen in iso-pentane precooled by liquid nitrogen, and finally transferred into liquid nitrogen. The biopsy specimens and sera were kept at –70°C until analyzed. All gastroscopies were performed during three consecutive years 2008-2010.

Immunohistochemistry

Cryostat sections (7 µm) were stained as previously described (77, 298). The slides were coded and evaluated in a random order. All sections, except the ones detecting eosinophilic endogenous peroxidase, were blocked by the glucose oxidase sodium azide method to quench endogenous peroxidase activity (299). Eosinophilic endogenous peroxidase was detected by just adding AEC-substrate. Mouse monoclonal antibodies with the following specificities were used: IgE, CD3, mast cells tryptase and CD11c. As a secondary antibody biotinylated horse anti-mouse IgG (H+L) was used followed by an avidin-biotin-peroxidase enzyme complex. The slides were developed by adding AEC substrate and counter stained with Mayer's Hematoxylin. Positively stained cells were counted by using a computer supported image analysis system Leica Q500MC. Cells in at least five 200 x fields from the villi and the basal lamina propria regions of the mucosa were counted by a blinded observer. The IgE, CD3 and tryptase positive cells were recorded as the number of stained cells/mm^2 of tissue. The density of CD11c+ dendritic cells was estimated as the relative stained area in at least five 100 x fields and expressed as percent stained tissue area.

Open challenges

In *study II*, all the patients in the PS-group underwent an open challenge with peanut and showed a negative outcome before they were included in the study. The open challenges were performed as part of the investigation according to EAACI position paper, and the total dose of peanut used for the open challenge was 10 g (158).

Allergen extracts used for the basophil activation test

The allergen extracts used in this study were: peanut (*Arachis hypogaea*, protein concentration 6 mg/ml); soy (*Glycine max*, protein concentration of 1.9 mg/ml); and birch (*Betula verrucosa*, protein concentration of 0.08 mg/ml) (Soluprick, ALK-Abelló, Hørsholm, Denmark). For the BAT, the allergen extracts were serially diluted in 10-fold steps from an initial 1/30 dilution of the Soluprick extract. The peanut extract was tested in twelve serial 10-fold dilutions, and the soy and birch extracts were tested in five serial 10-fold dilutions. For the serum samples from some patients, the BAT was repeated with additional dilutions.

Basophil activation test

Basophil activation was measured based on the up-regulation of CD63 on CD203c+ basophils observed in flow cytometry of blood samples collected in heparinized tubes. All the tests were carried out within 4 hours of blood sampling. To study the activation of basophils, the BasoFlowEx® Kit (EXBIO, Prague, Czech Republic) was used according to the manufacturer's recommendations. Briefly, 100 µl of heparinized whole blood and 50 µl of Stimulation Buffer were added to all the tubes. Subsequently, 5 µl of allergen solution (allergen extracts for peanut, soy and birch; Soluprick) were added to the samples. For the positive control, 10 µl of Stimulation Control [a cross-linking anti-IgE antibody mixed with a stimulating peptide, N-formyl-Met-Leu-Phe (fMLP)] were added. The tubes were gently vortexed and incubated at 37ºC for 15 minutes in a water bath, followed by mixing with 20 µl of Staining Reagent, which contained anti-CD63 FITC and anti-CD203c PE antibodies. After further incubation for 20 minutes on ice, 300 µl of Lysing Solution were added, and the tubes were re-incubated for 5 minutes at room temperature, followed by the addition of 4 ml of de-ionized water for 10 minutes. After centrifugation at 300 × *g* for 5 minutes, the supernatant fluid was removed and the pelleted cells were re-suspended in 0.4 ml PBS. Samples were analyzed in the BD FACSCanto II flow cytometer. The cut-off for

determining a positive test was set at 15% CD63-positive basophils, in line with the manufacturer's instructions. The gates were the same for all the tests conducted on an individual patient, although they were positioned individually for each patient. The level of basophil activation is expressed as %CD63^{+} basophils above the threshold set in the negative control.

Data collection procedures in Study III

The replies from food questionnaire (Hypersensitivity questionnaire, which is presented in Appendix of the Original Study III), regarding the reactions to different foods, were encoded for the different symptoms as presented in Table 1.

Encoded fields for milk, sour milk and cheese were added which were dissociated from the most relevant clinical symptoms for suspicious lactose intolerance as abd (abdominal pain), gas (flatulence) and dia (diarrhea / loose stools), or if lactose intolerance was specified in any free text field. Likewise, encoded fields were added for flour from wheat and flour from other cereal grains, in case of suspicious gluten intolerance (coeliac disease), that were dissociated from the clinical symptoms tir (tiredeness), abd (abdominal pain), gen (feeling of illness, tiredness), dia (diarrhea / loose stools), gas (flatulence) and/or urt (hives, urticaria), or if gluten intolerance was specified in any free text field. Using the above described procedure, most cases of suspicious lactose and gluten intolerance could be excluded from the calculations. Subjects with suspected asthma, based on the questionnaire, that could not be verified by the clinical examination as described in the previous section were excluded from the analyses.

Code	Meaning
Skin	Symptoms from the skin (urticaria, eczema, angioedema, flush, itching, tingling, skin pain, papules, redness etc.)
GI	Abdominal pain, oral symptoms, diarrhea, flatulence, reflux, vomiting, constipation
Airup	Symptoms from the upper airways –nose (rhinitis, nasal congestion, nasal itching, sneezing, red nasal papules) , eyes
Airlo	Lower airways – respiratory symptoms (heavy breathing, difficulty getting air, wheezing, cough, chest pressure, bronchospasm, hoarseness, mucus/saliva in the throat)
Circ	Palpation, fainting, dizziness
CNS	Headache, confusion
Oth	Other (e.g. ear itching, gallstone)
Not	Do not eat
Unk	Unknown, uncertain whether intolerant or not
Ana	Anaphylactic reactions
Gen	General symptoms such as tiredness, feeling ill

Table 1. Encoding for self-reported hypersensitivity reactions in food hypersensitivity questionnaire

Ethical considerations

Ethical permissions for all studies were received from the regional ethics review board in Gothenburg. Register numbers for permissions were:

Study I: 452-06

Study II: 591-10

Study III: 593-08

Statistics

Study I:

The values represent individual data points or means and median values. The statistical analyses were carried out by using SPSS Statistics 17.0. Data are reported as medians with interquartile ranges (IQR), and Mann–Whitney U-tests were used for statistical comparison between groups of patients. Data for each individual on cell counts in biopsies and IgE reactivity between the seasons was compared by Wilcoxon signed rank test. Correlations between different parameters within the same group were evaluated by Spearman's correlation coefficient or, after log-transformation, with Pearson's correlation coefficient. All tests were two-tailed and the level of significance was set to $P < 0.05$.

Study II:

The statistical analyses were carried out using the IBM SPSS Statistics 22.0 software. The values shown represent individual data-points or means and median values. For each patient, the basophil allergen threshold sensitivity was calculated as the lowest allergen concentration that was able to activate 50% of the basophils that were activated in the stimulation control (BAT AC50). The BAT AC50-value was calculated using a linear interpolation of the response to the allergen and is presented as the $\log_{10}$ value of the dilution factor, i.e., the higher the number the more sensitive is the patient. Data for BAT are reported as medians with interquartile ranges (IQR). Mann-Whitney U-tests were used for statistical comparisons of the groups of patients. Correlations between different parameters within the same group were evaluated by Spearman's correlation coefficient. The complete results of the most relevant and influential statistical correlations between the variables in PA-group and the PS-group obtained in the study are provided in Tables 1S and 2S in the Appendix.

Logistic regression analysis was performed to determine whether covariate diagnostic variables could be combined with the BAT results to achieve a more accurate diagnosis. All tests were two-tailed and the level of significance was set at $p<0.05$. Receiver operating characteristics (ROC) curve analysis was performed to calculate the optimal cutoff value of AC50 that corresponded to the highest specificity and sensitivity. Multivariate factor analysis (SIMCA-P+ software; Umetrics, Umeå, Sweden) was used to examine the relationships between individuals with severe peanut allergy or subjects sensitized to peanut (Y-variables) and the various parameters studied (X-variables). Projection to

latent structures discriminant analysis (PLS-DA) was implemented to examine whether allergic individuals compared with sensitized and control individuals could be discriminated based on the X-variables examined. Orthogonal partial least-squares discriminant analysis (OPLS-DA) was performed to correlate Y-variables and X-variables to each other in linear multivariate models. Variable influence on projection (VIP) values can be used to discriminate between important and unimportant predictors for the model. The OPLS-DA plot of the results (Fig. 9B) is based on X-variables with variable influences on projection values (VIP-values) ≥0.83, and the OPLS column loading plot in Figure 10A is based on VIP-values ≥0.88. In the OPLS analyses, the relative importance of each X-variable to the Y-variable is represented by column bars. The larger the bar and smaller the error bar, and the stronger and more certain is the contribution to the model. The most influential X-variables were used for subsequent statistical analyses.

Study III:

The statistical analyses were performed using SPSS 22.0 and Microsoft Excel 2007. Chi-squared test was used for the prevalence of self-reported symptoms as well as gastrointestinal symptoms to different foods among subjects with and without asthma. A p–value < 0.05 using Fischer's two tailed exact test was considered statistically significant. Correlations between different parameters within the same group were evaluated by using the Pearson's or Spearman's correlation coefficient. Tests were two-tailed and the level of significance was set to $P < 0.05$.

4 RESULTS

Study I:

The main results of the study are presented below. The S-group was patients with allergy to birch pollen and with gastrointestinal symptoms and the NS-group was birch pollen allergic patients without gastrointestinal symptoms.

In the S-group, half of the patients (n=10) reported symptoms only during the pollen season while the rest had subjective symptoms without any seasonal variation. The clinical characteristics of the subjects are shown in Table 2. The questionnaire used for grading the symptoms in the group of patients with gastrointestinal symptoms is presented in the Supplementary of the Original Study I.

	S (n=20)	NS (n=12)
Asthma	11	3
OAS	12	6

Gastrointestinal symptoms	**S (n=20)**
Pollen season-related symptoms	10
Symptoms all the time	10
Most frequent symptoms	**(%)**
Abdominal distension	78
Gases	72
Stomach pain	44
Diarrhea	44
Constipation	33
Pain in the lower bowel	28
Bowel cramps	28
Pain relief with defecation	22
Burning sensation in esophagus	22
Nausea	11
Pain related to meal	11

Table 2. The number of patients with asthma and OAS (oral allergy syndrome) and the frequency of the most frequent gastrointestinal symptoms. (S=with gastrointestinal symptoms, NS=no gastrointestinal symptoms, C=healthy controls).

Immunohistochemical parameters of duodenal mucosal

Allergic inflammation in the duodenal mucosa in birch pollen allergic patients compared to healthy controls

The numbers of IgE-positive cells, eosinophils, tryptase-positive cells and CD11c+ dendritic cells were significantly higher in S-patients compared with controls in the biopsies taken during the pollen season. Outside the pollen season, only IgE-positive cells and eosinophils were significantly higher in the S-patients. In the NS-patients the biopsies taken during the pollen season showed significantly elevated number of IgE-positive cells, eosinophils, CD3+ T cells, tryptase- positive cells and CD11c+ dendritic cells as compared with the controls. In this group only the frequency of IgE-positive cells and CD11c+ dendritic cells remained elevated outside the pollen season.

Intestinal allergic inflammation in birch pollen allergic patients with or without GI symptoms

There was no significant difference in the duodenal cell counts (mast cells, eosinophils, T cells, and CD11c+ dendritic cells) between the patient group with gastrointestinal symptoms (S) as compared to patient group without GI symptoms (NS). This was true both for samples taken during and outside the pollen season.

Seasonal variation in duodenal allergic inflammation

Patients with gastrointestinal symptoms (S-group):
During the birch pollen season there were significantly higher numbers of **IgE-positive cells**, **CD3+ T cells** and **CD11c+ dendritic cells** in patients with GI symptoms than in the same patients outside the pollen season. However, there was no difference between patients who experienced pollen season related gastrointestinal symptoms (n=10) compared to patients with symptoms not related to season (n=10) in this group.

Patients without gastrointestinal symptoms (NS-group):
Biopsies showed significantly higher numbers of **CD11c+ dendritic cells** (p=0.06) and **a tendency for elevated eosinophil counts** (p=0.050) **and IgE-positive cells** (p=0.060) during the pollen season as compared with the biopsy specimens taken off-season.

When examining the whole material including patients from both S- and NS-group, no significant differences could be seen in the duodenal cell populations when comparing patients with or without **asthma**, regardless of season.

In the **healthy controls**, there were no seasonal changes in any of the duodenal cell populations apart from the T cells that showed a slight but significant increase during the pollen season (p=0.034).

IgE reactivity against allergen components

The overall mean score of IgE reactivity to PR-10 proteins was significantly higher ($p < 0.001$) in the S-group 62.91 ISU (IQR 65.30; 95% CI 20.90-104.90) compared to NS-group 0.12 ISU (IQR 0.0; 95% CI −0.15-0.40). However, there were no significant differences in the IgE levels against the PR-10 proteins between the S and NS groups when analyzing the data separately for the different seasons. There was a trend towards an elevated median sensitization score in the S group to birch pollen related food 4.9 ISU (IQR 15.9; 95% CI 2.11-29.86) compared to NS group 2.75 ISU (IQR 9.65; 95% CI −0.5-18.5) in samples taken during the birch pollen season. The ISAC data with the detailed sensitization patterns of the patients are shown in the Supplementary Table of the study I.

Patients with GI symptoms (S-group)

In this group we noted significantly higher levels of IgE to the major birch pollen allergen **(rBet v 1)** ($p = 0.047$), hazel pollen **(rCor a 1.0101)** ($p = 0.014$), and apple **(rMal d 1)** ($p = 0.041$) in blood samples taken during the birch pollen season. There were no significant differences in the IgE reactivity to any of the other ISAC component regardless of pollen season. In the subgroup of patients with OAS there was a significantly elevated IgE reactivity to the pollen antigens both during and outside the pollen season for **rBet v 1** ($p = 0.020$, resp $p = 0.006$) and **rAln g 1** (alder) ($p = 0.010$, resp $p = 0.002$), as well as to the related food antigens , **rMal d 1** ($p = 0.044$, resp $p = 0.029$), **rPru p 1** (peach) ($p = 0.002$, resp $p = 0.002$), **rAra h 8** (peanut) ($p = 0.014$, resp $p = 0.022$), **rCor a 1.04** (hazelnut) ($p = 0.012$, resp $p = 0.004$). The IgE reactivity to PR-10 proteins in the serum samples correlated significantly with the numbers of eosinophils in biopsies taken during the pollen season and with the total IgE levels in serum both during and outside the pollen season ($r = 0.63$, $p = 0.004$ and $r = 0.67$, $p = 0.002$ respectively). A positive correlation

was also found between CD11c+ cells in biopsies taken during the pollen season and IgE for rCor a 1.0101 (r = 0.61, p = 0.006) and rAra h 8 (r = 0.50, p = 0.028). There was no correlation between IgE reactivity to components of the PR-10 proteins and the symptoms displayed by each patient neither during nor outside the pollen season.

Patients without GI symptoms (NS-group)

In this group of patients there was a significantly higher IgE reactivity to grass pollen **rPhl p 1** (p = 0.010) and apple **rMal d 1** (p = 0.018) in blood samples taken during the birch pollen season and in rAln g 1 (p = 0.002), nAct d 8 (p = 0.018) and rGly m 4 (p = 0.017) in samples taken outside the birch pollen season. In the subgroup of OAS patients we found a significantly elevated IgE reactivity to some pollen and pollen related components like **rCor a 1.0101** (p = 0.022), **rPru p 1**, **rAra h 8** (p = 0.036) in samples taken during the pollen season, and to **rCor a 1.0101** (p = 0.013), to **rPru p 1** (p = 0.037) as well as **rAra h 8** (p = 0.020) in samples taken off-season.

Healthy controls

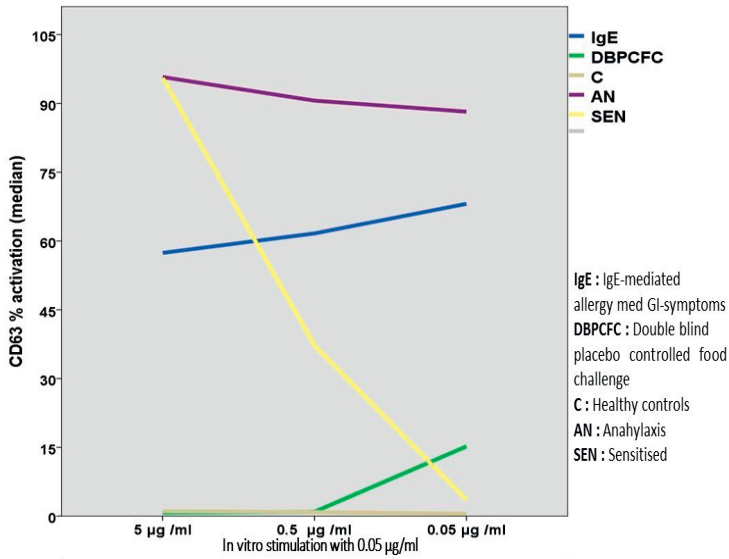
None of the healthy controls showed IgE reactivity to any of the allergens tested.

Study II:

The evaluation of the BAT as a tool for the diagnosis of food allergy was examined in a pilot study before the more extensive *Study II*.

In this pilot study BAT was used for the diagnosis of allergy to different foods and between different groups of patients and control subjects. The groups comprised of 10 patients with IgE-mediated anaphylaxis to different foods (AN), 10 patients with IgE-mediated food allergy with gastrointestinal symptoms (IgE), and 9 patients with non-IgE mediated food allergy with gastrointestinal symptoms, proven with DBPCFC (DBPCFC). As reference or control subjects we used 10 patients who were IgE-sensitized to different foods (SEN) and 10 completely healthy non-allergic individuals (C). The patients of all groups besides the AN-group, were challenged with the food stuff they were allergic or sensitized to, and the patients in the C-group where challenged with the most common foods in order to be available for comparison with the other patient groups. The BAT was used for analysis both before and 2h as well as 24h after the food challenge. In addition the basophils were stimulated with three different concentration of the relevant food allergen (5µg/ml, 0.5µg/ml and 0.05µg/ml respectively). Basophils were identified by the marker 203c and their activation was measured by up-regulation of CD63 in flow cytometry. The foods used in this study were: cow's milk, hen's egg, wheat, peanut, soy, codfish, shrimp and rye.

The results did not show any significant activation of the circulating basophils after oral challenge with the different foods, despite that all food allergic patients got symptoms when challenged with the offending food. The patients in the AN- and IgE-group showed significantly stronger reaction in *in vitro* basophil stimulation compared to the other groups. By this pilot study, we concluded that, the BAT in vitro, may be used as a complement in diagnosing IgE-mediated food allergies but it is still blunt in discriminating between patients with true clinical allergy and sensitized subjects. However, the BAT is a promising tool in predicting the outcome of a food challenge in patients with suspected anaphylaxis. This pilot study was presented as abstract in the European Academy of Allergy and Clinical Immunology in London in June 2012. The results are presented below in Figure 8.



	AN	IgE	C	SEN	DBPCFC
AN	--	*	*	*	*
IgE	*	--	*	*	
C	*	*	--	*	*
SEN	*	*	*	--	
DBPCFC	*		*		--

* p < 0.05

Figure 8. The percentage of CD63+ basophils, after stimulation with 0.05µg/ml of the relevant food allergen in the different groups of patients and control subjects The Table presents the results from the comparison between the different groups of patients and reference/control subjects to each other, for the activation of CD63+ basophils, using the Mann-Whitney U-test (*p<0.05).

In *study II*, PA was the patients with severe peanut allergy and PS was the peanut sensitized patients. The main results of *the Study II* are presented below.

The demographic and clinical characteristics of the patients and control subjects are shown in Table 3.

	PA (n=47)	PS (n=22)	C (n=22)
Men	25	7	5
Women	23	15	11
Age (mean)	25	28	33
Sensitization to peanut (sIgE)	47	20	-
Sensitization to peanut (SPT)	47	18	-
Sensitization to birch (sIgE)	36	20	-
Sensitization to birch (SPT)	34	18	-
Sensitization to soy (IgE)	34	11	-
Sensitization to soy (SPT)	18	13	-
Asthma	31	11	-
Immunotherapy (birch pollen)	5	3	-
Open peanut challenge during allergy investigation	8	22	-
Peanut avoidance	47	7	-

Table 3. Clinical and demographic features of the patient groups included in the study (PA=peanut allergic patients, PS=peanut sensitized patients, C=healthy controls).

Peanut allergy can be distinguished from peanut sensitization in a multiple regression model that includes conventional tests and BAT to peanut

The multiple regression model discriminates patients with severe allergy to peanuts from patients sensitized to peanuts and from healthy control subjects according to the distribution as presented in Figure 9A. In addition, the model depicts the most associated variables between patients with severe allergy to peanuts and patients sensitized to peanuts as shown in Figure 9B.

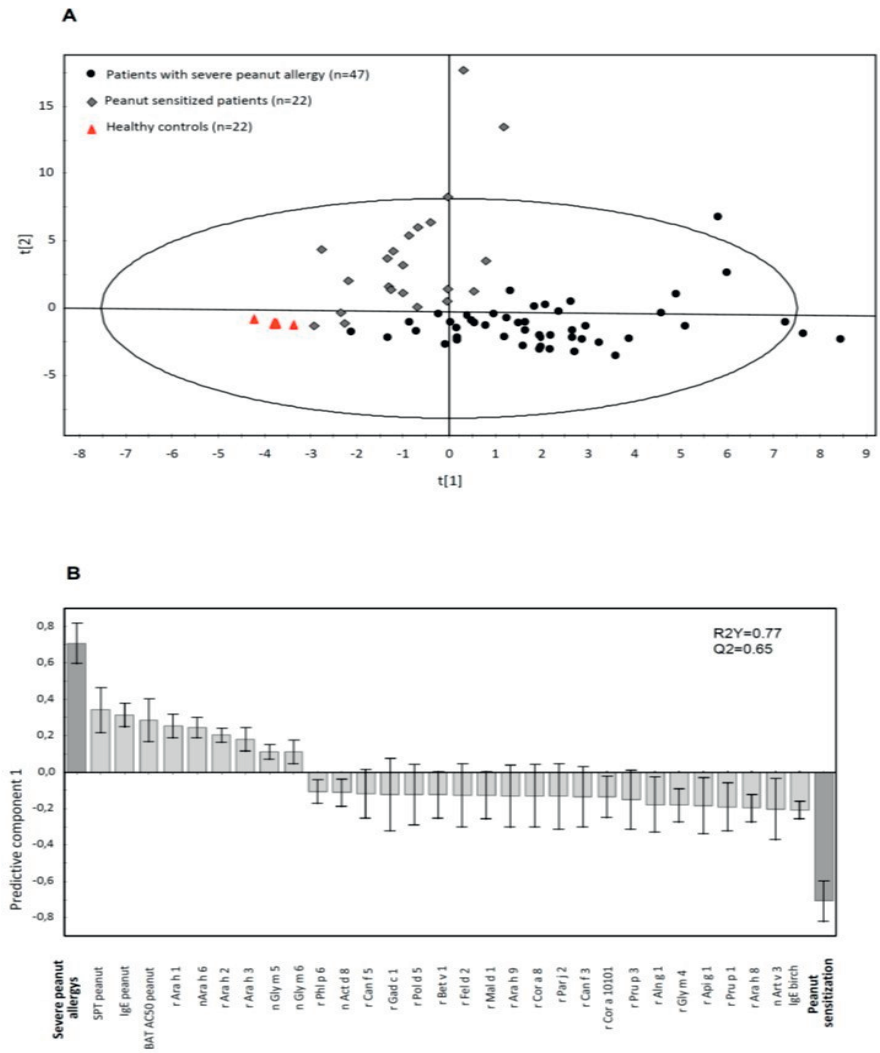


Figure 9. **(A)** PLS-discriminant analysis score scatter plot showing the separation between patients with severe allergy to peanuts (black dots, n=47) and patients sensitized to peanuts (green diamonds, n=22). All healthy controls are clustered in the lower left quadrant (red triangles, n=22). **(B)** OPLS-discriminant analysis column loadings plot depicting the associations between patients with severe allergy to peanuts and patients sensitized to peanuts. X-variables represented by a bar pointing in the same direction as severe allergy to peanuts (located to the far left) are positively associated. The PLS-DA column plot is based on X-variables with VIP-values ≥0.83. R2Y indicates how well the variation of Y is explained, while Q2 indicates how well Y can be predicted.

Severe allergy to peanuts was positively associated with SPT to peanut, specific IgE to peanut, BAT AC50 to peanut and several Ara h components, i.e. 1, 6, 2 and 3 (Figure 10).

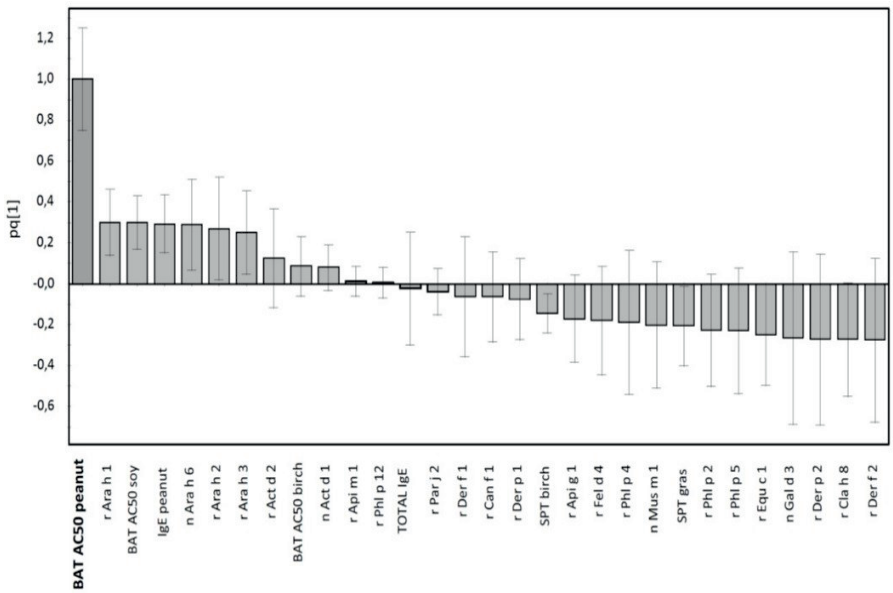


Figure 10. OPLS column loading plot showing the X-variables most associated with BAT AC50-value for peanut within the PA group. X-variables represented by a bar pointing in the same direction as AC50 peanut (located to the far left) are positively associated, whereas variables in the opposite direction are inversely related. The OPLS plot is based on X-variables with VIP-values ≥0.88. R2Y indicates how well the variation of Y is explained, while Q2 indicates how well Y can be predicted. (PA=patients with severe peanut allergy, PS=peanut sensitized patients)

Basophil activation test results for peanut-allergic versus peanut-sensitized patients.

The median BAT AC50 value obtained for peanut was significantly higher for the PA-group at 6.84 (IQR 4.50) than for the PS-group at 3.55 (IQR 4.15) (p<0.001). In the PA-group, there were 3 (6%) non-responders to peanut and 5 (23%) in the PS-group. No significant differences were noted comparing the median BAT values for birch or soy between the two groups. Basophils from

healthy controls did not respond to any of the allergens tested.

Does peanut BAT outcome correlate with other allergy parameters in peanut-allergic patients?

In the PA-group, there was a positive correlation between the BAT AC50 to peanut and BAT AC50 to soy (r=0.413, p=0.004) but no correlation to the BAT AC50 for birch (r=0.161, p=0.280). Interestingly, in this group, we found a rather weak correlation between the BAT AC50 for peanut and the levels of IgE directed against the individual peanut components rAra h 1 (r=0.314, p=0.032), rAra h 2 (r=0.291, p=0.047), rAra h 3 (r=0.289, p=0.049), and nAra h 6 (r=0.347, p=0.017), and surprisingly, no correlation with the SPT or IgE level to peanut nor with total serum IgE.

Basophil activation test to soy and birch in peanut allergic vs peanut sensitized patients

In the PA-group, there were positive correlations between the BAT AC50 for soy and specific IgE to soy (r=0.585, p<0.001), as well as specific IgE directed against peanut (r=0.584, p<0.01). There was a similar correlation to the individual component nGly m 6 (r=0.583, p<0.01) but a weaker correlation to nGly m 5 (r=0.391, p<0.01), as well as to the peanut components rAra h 1 (r=0.508 p<0.001), rAra h 2 (r=0.476, p<0.001), rAra h 3 (r=0.661, p<0.001), and nAra h 6 (r=0.484, p=0.001).

In the PS-group, there were positive correlations between the BAT AC50 for peanut and the BAT AC50 for soy (r=0.689, p<0.01), but also the BAT AC50 for birch (r=0.735, p<0.01). In this group, the BAT reactivity to peanut correlated only with IgE to rAra h 8 (r=0.479, p=0.024) and rGly m 4 (r=0.638, p=0.01). Interestingly, in this group, a correlation was also found between the BAT AC50 for soy and the level of IgE to peanut (r=0.470, p=0.027), as well as to the soy component rGly m 4 (r=0.447, p=0.037).
A complete list of the significant statistical correlations observed between the studied variables in both groups is provided in Supplementary Tables 1S and 2S in the Appendix, for the PA- and PS-group, respectively.

Can BAT discriminate between peanut-allergic and peanut-sensitized patients?

To determine a cut-off level for reactivity to peanut in the BAT, so as to distinguish between the patients in the PA-group and PS-group, ROC curves

were applied. They revealed that an optimal sensitivity of 79% and a specificity of 86% could be obtained at a BAT AC50 of 5.27, which would allow the diagnosis of patients with severe peanut allergy (AUC 0.862). With SPT to peanuts, the highest sensitivity of 83% and highest specificity of 82% were obtained for a wheal diameter of 3.5 (AUC 0.910), and the IgE to peanuts showed a sensitivity of 81% and specificity of 91% at an IgE level of 11.5 kU/L (AUC 0.922). The parameters that showed the greatest power for distinguishing the two groups in the ISAC assay were IgE to rAra h 2 [sensitivity of 91.5% and specificity of 100% at an IgE level of 0.75 ISU (AUC 0.957)], and nAra h 6 [sensitivity 100% and specificity 100% at a level of 1.16 ISU(AUC 1.0)].).

The results concerning the IgE reactivity between the different groups are presented in Table 4.

IgE-reactivity (median)	PA (n=47)	PS (n=22)	C (n=22)
Total IgE	380.0 (3-1800)	200.0 (13-4600)	17.5 (2-140)
IgE peanut	64 (0.55-100)	2.45 kU/L (0.1-15)	<0.35 (0.0-0.05)
IgE birch	6.1 (0.0-100)	25.0 kU/L (0.0-100)	<0.35 (0.0-0.24)
IgE soy	1.4 (0.0-40)	0.36 kU/L (0-40)	<0.35 (0.0-0.14)
rAra h 1	30.55 (0.0-138.71)	<0.3	<0.3
rAra h 2	10.75 (0.0-1117.58)	<0.3	<0.3
rAra h 3	5.38 (0.0-64.40)	<0.3	<0.3
nAra h 6	23.55 (1.21-178.24)	<0.3	<0.3
rAra h 8	<0.3	1.60 (0.0-17.80)	<0.3
rAra h 9	<0.3	<0.3	<0.3
rGly m 4	<0.3	1.11 (0.0-27.88)	<0.3
nGly m 5	<0.3	<0.3	<0.3
nGly m 6	1.62 (0.0-53.60)	<0.3	<0.3
rBet v 1	8.85 (0.0-100)	24.96 (0.0-168.42)	<0.3

Table 4. The median (range) total IgE and specific IgE expressed in kU/L. The median (range) for rAra h 1,2,3,6,8,9 which are the recombinant components for peanut, rBet v 1 for birch pollen and rGly m 4, nGly m 5, rGly m 6 for soy are expressed in ISU (ISAC Standard Units). (PA= patients with severe allergy to peanuts, PS=peanut sensitized patients, C=healthy controls).

The frequency of IgE sensitization to allergen components is presented in Table 5.

Peanut allergens components	PA (n=47)	PS (n=22)
rAra h 1	40	0
rAra h 2	43	0
rAra h 3	32	1
nAra h 6	47	1
rAra h 8	15	15
rAra h 9	0	2

Table 5. IgE sensitization frequency to allergen components (>0.35 ISU) between patients allergic to peanuts and patients sensitized to peanuts. (PA=peanut allergic patients, PS=peanut sensitized patients)

Study III:

The main results of the study are presented below.

From the 1527 subjects finally included in the study, 583 (38.2 %) had asthma while 944 (61.8 %) had no asthma ($p<0.001$). Among the subjects with asthma 192 (32.9 %) were sensitized to birch pollen compared with 119 (12.6 %) among non-asthmatic subjects ($p<0.001$).

Prevalence of food hypersensitivity in adults with asthma compared to adults with no asthma.

Subjects with asthma reported a considerably higher prevalence of adverse reactions to food compared to those without asthma 53.1 % vs. 29.8 % ($p < 0.001$). When symptoms from suspicious lactose and gluten intolerance were excluded, asthmatics still reported more adverse reactions to food compared to non-asthmatics, 51.3% vs. 28.2 % ($p < 0.001$).

Association between adverse reactions from specific foods and asthma

Subjects with asthma most commonly experienced adverse reactions (including all types of symptoms) to hazelnut (20.5 %), apple (17.5 %), kiwi (14.3 %), walnut (12.8 %), milk (11.5 %), peach (10.7 %), brazil nut (9.8 %), almond (9.5 %), nectarine (9.3 %), pear (8.9 %), plum (8.8 %), cherry (8.7%), wine/beer (8.0 %), peanut (7.0 %), shellfish (6.5 %), carrot (6.4 %), strawberry (6.4 %), and apricot (6.3 %). Concerning the staple and dairy food items, subjects with asthma experienced adverse reactions most commonly against milk (including subjects with suspected lactose intolerance, 11.5 %), shellfish (6.5 %), sour milk/yogurt (6.25 %), cheese (4.5 %), egg (3.3%), fish (2.9 %), soy (1.4%), wheat (including subjects with suspected gluten intolerance, 3.23 %) while about 1.4 % to other flours.

Association between self-reported food-related gastrointestinal symptoms and asthma

Subjects with asthma also report significantly more gastrointestinal symptoms to hazelnut (13.0% vs 5.2%, $p<0.001$), apple (11.4% vs 6%, $p<0.001$), milk (10.4% including subjects with suspicious lactose intolerance vs 5.7%, $p<0.01$), kiwi (9.7% vs 5.3%, $p<0.01$), peach (8.3% vs 2%, $p<0.001$), plum (6.75% vs 2.2%, $p<0.001$), nectarine (6.7% vs 1.3%, $p<0.001$), pear (6.4% vs 2.4%, $p<0.001$), cherry (6.2% vs 2.4%, $p<0.001$) followed by walnut (5.9% vs 3.0%,

p<0.01), fried/fat food (5.7% vs 3.3%, p<0.05), sour milk/yoghurt (5.6% vs 2.8%, p<0.01) and almond (5.45% vs 2.3%, p<0.01) compared to non-asthmatics.

IgE sensitization for the most common foods among asthmatics and non-asthmatics

When assessing the sIgE-sensitization profiles for the most-common foods in panels fx1 and fx5, we observed that subjects with asthma are generally more frequently sensitized to the food items tested compared to non-asthmatics (38.2 % vs 13.9 %, p < 0.001). More specifically, when comparing asthmatics with non-asthmatics it was found that subjects with asthma were significantly more frequently sensitized to hazelnut (31.8 % vs 11.2 %, p < 0.001), peanut (9.1 % vs 4.3 %, p < 0.001), almond (6.6 % vs 2.4 %, p < 0.001), milk (6.0 % vs 1.6 %, p < 0.001), wheat (5.5 % vs 1.8 %, p < 0.001), egg (5.3 % vs 1.4 %, p < 0.001), soy (3,5 % vs 1.1 %, p = 0.003), brazil nut (2.2 % vs 0.4 %, p = 0.003), and fish (1.3 % vs 0.0 %, p = 0.001). Hazelnut is one of the birch pollen-related foods and IgE to hazelnut correlated strongly with IgE to birch in the asthmatic subjects (r=0.904, p<0.001) as well as in non-asthmatic subjects (r=0.920, p<0.001). When looking for possible correlations between IgE-sensitization and self-reported symptoms for the most common foods, we observed the highest correlation between IgE and self-reported symptoms for hazelnut (r=0.496, p<0.001) in the asthmatic group as well in the non-asthmatic adults (r=0.499, p<0.001). IgE sensitization to birch also correlated with self-reported symptoms from hazelnut both in subjects with and without asthma, although slightly weaker (r=0.455, p<0.001 resp. r=0.472, p<0.001).

Seasonal variation of gastrointestinal symptoms in subjects with and without asthma

Asthmatics experienced more symptoms from the gastrointestinal tract during the spring (6.7 % vs 2.2 %, p < 0.001), summer (5.1 % vs 1.9 %, p = 0.001) and autumn (5.9 % vs 3.2 %, p = 0.013), but not during the winter compared to non-asthmatics. In addition, asthmatic subjects with IgE reactivity to birch pollen more frequently report gastrointestinal symptoms compared to birch pollen sensitized subjects without asthma during the spring (5.7 % vs 0.8 %, p = 0.034), summer (4.2 % vs 0.0 %, p = 0.026) and autumn (3.7 % vs 0.0 %, p = 0.046).

5 DISCUSSION

Allergic gastrointestinal inflammation in patients allergic to birch pollen

The results of the *study I*, show that birch-pollen allergic patients with gastrointestinal symptoms, display a significant intestinal allergic inflammation with elevated numbers of eosinophils, IgE-positive cells, CD3+ T cells and CD11c + dendritic cells during the pollen season. This confirmed previous findings (67). The design of the present study with a larger patient sample than in the previous study, allowed us to discriminate between patients with or without subjective GI symptoms and revealed that the intestinal allergic inflammation was obvious also in asymptomatic patients. Also, in these patients, the pathology was aggravated during the birch pollen season. Furthermore, in both patients with and without GI symptoms, we observed elevated levels of IgE to most of the PR10-proteins during the pollen season. This was particularly pronounced in patients with OAS, indicating that cross reactions to birch related food may play a role in their pathology, which is supported by the "trigger foods" causing GI symptoms in OAS (300). In addition, we observed that patients with allergic asthma were more prone to duodenal allergic inflammation as compared with non-asthmatic patients. The reason for this is unclear but could be due to a more severe allergy with greater engagement of the common mucosal immune system.

An elevated production of IgE antibodies against pollen allergens (301) during the birch pollen season is well established, but there are very few studies exploring the sensitization pattern to birch pollen related foods in relation to the birch pollen season (302). Interestingly, high prevalence of clinical reactions to fruits and vegetables has been shown in birch pollen-sensitized patients with even higher prevalence in multi pollen-sensitized patients, which supports the notion that ingestion of pollen-related foods may act as an eliciting factor for allergic symptoms from different organs (303). We found that IgE levels against the major birch allergen Bet v 1 as well as birch pollen related food items, are clearly increased during the birch pollen season in both groups of birch pollen allergic patients. In addition we observed that the IgE levels to some of the birch-pollen related foods like apple and peach, but also peanut, were significantly higher in patients with OAS. These findings support the hypothesis that ingestion of food items that are related to birch pollen might have a significant role in the allergic inflammation of the intestinal mucosa (72, 77). This may suggest that ingestion of birch pollen related food items during the birch pollen season could precipitate the onset of the gastrointestinal symptoms observed in pollen allergic patients. In support of this a recent study by Pickert

et al. showed that 81% of patients with birch pollinosis and GI-symptoms, display a wheal and flare reaction in the gastrointestinal mucosa after colonoscopic allergen provocation with Bet v 1. Interestingly, a similar mucosal reaction was observed in 22% of birch pollen allergic patients with pollinosis who did not experience any GI-symptoms (304).

In study I the patients in the S-group were found to have a continuous intestinal eosinophilia not related to the pollen season, which may indicate that they react to ingested pollen related food. This is supported by the positive correlation between eosinophil counts in in-season biopsies and the IgE reactivity to the PR-10 proteins during the pollen season. It is interesting though that birch-pollen allergic patients without gastrointestinal symptoms have a significantly increased intestinal eosinophilia during the pollen season. This may suggest a reaction to the pollen itself rather than pollen related food items, with a dissemination of the inflammation within the common mucosal immune system. A similar phenomenon has been observed in patients with eosinophilic esophagitis, where signs of a generalized subclinical eosinophilic inflammation at mucosal sites were part of the pathology (69, 70). Eosinophilic infiltration of the esophageal mucosa in patients with respiratory tract allergy has also been noted during the period of pollen allergy symptoms (70).

A number of clinical studies suggest a possible link between atopy and the augmentation of gastrointestinal symptoms during the birch pollen season in patients allergic to pollen(65, 305, 306), but few have addressed the cause of these GI symptoms (304). Interestingly, in the present study the most frequent GI symptoms reported were abdominal distension, gases, pain, diarrhea, and constipation, which are common symptoms in patients diagnosed with IBS. Mast cells have been proposed to be included in the pathogenesis of IBS and increased numbers of mast cells have been found in intestinal mucosal biopsies in patients with IBS(307-309). In line with these results we found a significantly higher number of IgE positive as well as tryptase-positive cells in the group of patients with GI symptoms compared to controls. In a more recent study examining both allergic and non-allergic patients with IBS, there was a higher number of eosinophils in the intestinal biopsies from patients with both atopy and IBS (66). These results indicate that a number of patients with an allergic inflammation in the GI tract are diagnosed with IBS, which may hinder a correct diagnosis of their illness.

BAT, a diagnostic tool for the severe peanut allergy in adults

In the *study II*, we show that BAT is useful as a complementary tool for the diagnosis and evaluation of severe peanut allergy in adults. The study clearly shows that basophil reactivity is significantly higher in patients with a history of severe allergy to peanuts (PA), as compared with peanut-sensitized (PS) patients; with a ROC area under the curve of 0.862 and at a BAT AC50 value of 5.27 the BAT shows a specificity of 86% and a sensitivity of 79%. The BAT AC50 value of 5.27 corresponds to a concentration of peanut antigen of 1.8 ng/ml being used to stimulate the basophils. Interestingly, the BAT AC50 value for the PA-group only weakly correlated with the ISAC value for the peanut components rAra h 1, rAra h 2, rAra h 3, and rAra h 6, which indicates that these two tests complement each other. This suggests that BAT can serve as a complementary diagnostic tool to the conventional investigations with SPT and specific IgE for patients with suspected severe peanut allergy.

Recently, a study conducted by Santos et al. (208), in which children with a history of anaphylaxis to peanut were compared with peanut-sensitized children, showed that the BAT could distinguish children with severe peanut allergy from children sensitized to peanuts with a sensitivity of 97.6% and a specificity of 96% (208). In that study, the majority of the subjects underwent an open challenge with peanut in addition to the BAT and conventional allergy tests. These results are in the line with the results of the present study. Other research groups have also proposed the BAT as a diagnostic tool for peanut allergy. Homsak et al. (310) reported that BAT reactivity values were higher in children who experienced severe reactions than in children with milder reactions. Glaumann et al. (207, 311) showed that children who reacted to peanuts in a DBPCFC had a higher BAT reactivity than non-reactors. In the present study, none of the patients with a history of anaphylaxis or very high IgE titers was investigated with an open challenge, so these results could not be confirmed.
The importance of rAra h components for predicting true peanut allergy is well documented in studies conducted on children (145-147, 312). Accordingly, in the present study, the patients who were diagnosed with severe peanut allergy (PA) also showed significantly higher levels of IgE to the peanut allergen components rAra h 1, rAra h 2, rAra h 3, and nAra h 6, as compared with the peanut-sensitized patients (PS). However, recently it was shown that IgE to rAra h 2 was the best predictor of clinical peanut allergy in peanut allergic patients, but rAra h 2 reactivity alone could neither discriminate between mild or severe peanut allergy nor could its absence exclude peanut allergy in an adult population (149). It has been suggested that in children, measurements of rAra h2 and nAra h 6 (as homologs) should be adequate as complementary tests

(147), while in the study of Bindslev-Jensen et al. (156), which was carried out in a mixed population of children and adults, it was found that IgE reactivity to rAra h 2 needed a cut-off of 1.63 kU/L to reach a specificity of 100% and sensitivity of 70%. IgE reactivities to these peanut components have been proposed as a complementary test to provide support for a diagnosis of suspected severe peanut allergy (145-147, 312). However, when analyzing the reactivity to birch we clearly see that peanut-sensitized, non-anaphylactic patients (PS) show a significantly higher level of specific IgE to birch, rBet v 1, and rAra h 8 which indicates that they are sensitized to peanut due to a cross-reaction between birch pollen and peanuts (148).

It has been proposed that individuals with severe allergy to peanut may develop a clinical sensitization to legumes and *vice versa,* however there is little evidence to support the notion that patients who are allergic to peanut develop an allergy to soy because of the cross-reactivity between the proteins in the two allergens (313, 314). On the other hand, patients who are allergic to birch pollen may also develop a clinically low-grade reactivity to soy protein due to cross-reactivity that exists between rGly m 4 in soy and the major birch pollen protein rBet v 1 (315-317). For patients with severe reactions to soy, it has been suggested that they are sensitized to the proteins nGly m 5 and nGly m 6 (318). This is supported by the results obtained in the present study, in which we observed that IgE directed against both nGly m 5 and nGly m6 correlated with the BAT AC50 for soy, specific IgE to soy extract, and SPT to soy. These results suggest a clinical allergy to soy in the PA-group. It is interesting to note that the reactivities to nGly m 5 and nGly m 6 also correlated with the levels of specific IgE to peanut and peanut recombinant allergens rAra h 1, rAra h 2, rAra h 3, and nAra h 6 in the PA-group, which suggests that sensitization to soy exerts an important clinical impact. This may explain why patients with peanut allergy also show adverse reactions to soy. In contrast, in the PS-group, rGly m 4 correlated with the BAT AC50 for birch, specific IgE for birch, rBet v 1, and rAra h 8, which can be attributed to the co-existent allergy to birch pollen. The component rGly m 4 did not correlate with the peanut components rAra h 1, rAra h 2, rAra h 3, and nAra h 6 or with specific IgE to peanut, which implies that reactivity to this component is not related to true peanut allergy. In the PS-group, it is clear that rGly m 4 is correlated with specific IgE to birch, rAra h 8, and rBet v 1, which confirms the data concerning cross-reactivity between birch pollen and this soy protein (317). In addition, our study clearly shows higher BAT AC50 values for soy in the PA-group than in the PS-group, which supports the idea that patients with severe allergy to peanut also have developed a more severe allergy to soy.
It is worth mentioning that in one patient from the PA-group who had high IgE titers to peanut (92 kU/L) and near-maximal activation of basophils to peanut

(ten serial 10-fold dilutions), there were maximal basophil responses to both soy and birch (ten serial 10-fold dilutions). Interestingly, this patient had low IgE titers for soy (0.97 kU/L) and birch (0.55 kU/L), which implies that the BAT may be a sensitive method for detecting potential anaphylactic responses to allergens not identified by IgE reactivity.

The high levels of sensitivity and specificity of the BAT in identifying individuals with clinically important IgE-mediated food allergy were confirmed in a previous study in which patients allergic to birch with oral allergy syndrome (OAS) to apple were compared with birch-allergic patients without OAS to apple (200).
In the present study, it is shown that the BAT AC50 for peanut does not correlate with the levels of IgE to the peanut allergen components, which suggests that the BAT can identify patients who are allergic to peanuts and who are not diagnosed with the conventional IgE-tests. A combination of SPT, specific IgE, recombinant allergens of peanuts, and the BAT may be optimal for securing an accurate diagnosis, as supported by a recent report from Spain (319).

Food allergy and asthma in adults

In *study III*, the subjects with asthma more frequently reported adverse reactions to foods compared to non-asthmatics (53 % vs 30 %) , and patients with asthma more frequently showed IgE reactivity to the most common foods. These results are in line with data from a previous study by Woods et al. in which it was suggested a positive association between IgE sensitization to foods and asthma or allergic disease (320). The data was supported also with the sensitisation patterns of specific-IgE for the most common foods found in the present study. We also show that asthmatics reported symptoms from the GI-tract in a greater frequency compared to non-asthmatics and the most common foods causing self-reported symptoms were nuts, fruits, milk dairy products, alcohol, peanuts and shellfish. The main allergens found in the reported fruits and nuts are related to birch pollen, which carry allergens with known cross-reactivity of PR-10 allergens. This may explain the high prevalence of adverse reactions to these foods, since birch pollen sensitization is very common in Sweden (321). This connection was confirmed in the present study where 32.9% of the asthmatics and 12.6% of the non-asthmatics were sensitized to birch pollen. When testing the subjects included, with the ImmunoCAP allergen panels for the most common staple foods and nuts (fx1 and fx5), subjects with asthma are more frequently sensitized to hazelnut, peanut, almond and milk than non-asthmatics, which is mainly in accordance with the results from self-reported symptoms in this study. However, the correlation between IgE sensitization to specific food items and the symptoms they cause are rather low, but still significant.

Concerning the staple foods, we show that asthmatic subjects more frequently report symptoms from egg, fish, milk (2.35 % when excluding lactose intolerance symptoms), and wheat (when excluding gluten intolerance symptoms) as well as soy. When trying to exclude subjects with suspected intolerance to gluten and/or lactose, the risk of losing some subjects with true allergy is inevitable, however the difference between asthmatics and non-asthmatics still remain. These results are in the line with previous reports from a Swedish epidemiological survey by Eriksson et al concerning self-reported food hypersensitivity in north Europe (322). Interestingly subjects with asthma report significantly more symptoms in high rates after alcohol ingestion as from wine/beer compared to non-asthmatics (7.97% vs 5.41%, $p<0.001$), which is supported by results from previous reports (323-325). When taking into consideration the IgE sensitization to birch pollen, we observe that among both asthmatics and non-asthmatics, birch-related foods are the most common causatives for adverse reactions with hazelnut in the first place (20.5% and 7.2 % respectively) followed by apple (17.5% and 7.15 % respectively) and other birch related fruits and nuts.

It is worth to comment that the prevalence of allergic asthma is much higher in the pediatric and adolescent population (326) and the prevalence of non-allergic asthma equals at about 40 years of age and thereafter the non-allergic asthma dominates (327-329). This may suggest that IgE-sensitization to the different foods and even other allergens may be stronger related to allergic asthma in the pediatric population and less so in adults. However as shown in the present study, also adult asthmatics have a high frequency of adverse reactions to foods that correlate with IgE sensitization.

The possible seasonal variation in gastrointestinal symptoms may be related to the pollen season where exposure to pollen may increase the reactivity after the ingestion of pollen related food items (321), which could be aggravated by the increased intestinal permeability seen in asthmatic patients (83) as well as in patients with atopy and IBS (66). In two other studies, it was demonstrated that asthmatics with allergy to birch pollen experience more symptoms from the gastrointestinal tract, which resemble irritable bowel syndrome (IBS)-like symptoms, during the pollen season (67, 68). It has also been shown that atopic subjects with IBS and self–reported food hypersensitivity had more severe gastrointestinal symptoms when compared to non-atopic subjects with IBS (66). Interestingly, besides the reported symptoms from the birch-pollen related foods, asthmatics reported more gastrointestinal symptoms to fried/fat food, foods rich in carbohydrate, wine/beer, legumes and spices which would signify that these patients may more frequently suffer from IBS (64, 65).

Limitations of the studies

One possible limitation in *study I* is that the patients were recruited during three consecutive years with variable severity of the seasonal pollen exposure, and that the individual natural exposure to pollen also varies according to living habits. However, the birch pollen seasons during the study years 2008–2010 were of representative severity for the study area without extreme variations.

A limitation of *study II* was that patients with suspected severe allergy to peanut could not be investigated with an open challenge for ethical reasons. Therefore, a correlation between the BAT outcome and the present clinical anaphylactic status of patients is not available. As there are still very few studies investigating the BAT as a diagnostic tool in adults with allergy to peanuts, more studies are needed to establish its diagnostic potential to predict severe reactions to peanuts.

Finally, concerning the *study III*, self-reported food intolerance yield a much higher prevalence compared to prevalence from performed food challenges and IgE data for food allergies from previous studies mainly in the pediatric population, but the comparison between asthmatics and non-asthmatics should still be valid, since we have no reason to believe that the self-reporting accuracy differs between these two groups. It would also have been of great value to have asked specifically for lactose and gluten intolerance, and not only get input from the free text fields. Nevertheless, the reported symptoms do affect the subjects, whether it is a true allergy or not. The large number of participants in the study make the findings reliable and fascinating as there are very few studies examining the relation between food hypersensitivity and IgE sensitization to the most common foods in adults.

6 CONCLUSION

In the first study, we have shown that, regardless of subjective gastrointestinal symptoms, patients allergic to birch pollen have clear signs of an ongoing allergic inflammation in their intestinal mucosa, which is aggravated during the pollen season. Furthermore, patients who experience GI symptoms show somewhat elevated IgE levels to PR-10 proteins compared to the asymptomatic patients, which could be associated with the intake of birch pollen related food items.

In the second study, we found that the BAT is helpful in determining severe peanut allergy and may be used as a complementary diagnostic tool to improve the accuracy of diagnosis of peanut allergy in adults, without the need for supplementary investigations involving an open challenge with peanuts. Furthermore, the BAT may be useful in revealing a hidden yet serious allergy to soy in patients with peanut allergy.

The novelty of the last study, is that it examines the relation between self-reported hypersensitivity and IgE-sensitization for the most common foods and in adult asthmatics and non-asthmatics as well as the relation between asthma and gastrointestinal symptoms due to different foods in adults for which the available data are still very scarce. In conclusion, the prevalence of both self-reported symptoms and IgE sensitization to most common foods were much higher among asthmatics compared to non-asthmatics, both in total and for the food items individually. Hazelnut and other birch pollen related foods most commonly induced gastrointestinal symptoms in asthmatics and we propose that one important factor that may explain this result is the high frequency of sensitization to birch pollen in asthmatic patients.

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REFERENCES

1. William M. The Gold-Headed Cane. New York: Hoeber. 1926:223.
2. Lundy L. A brief history of food allergies. ©2007.
3. Hare F. The Food Factor Disease. London: Longmans. 1905;I, II.
4. Schofield AT. A case of egg poisoning. Lancet. 1908:716.
5. Schloss O. A case of food allergy: Indiosyncracy to eggs, almond and oats due to anaphylaxis. Arch Paediat. 1919;29:219.
6. Coca A. The Pulse Test. New York: Arco. 1956.
7. Rinkel HJR, T. G.; Zeller, M. Food Allergy. Springfield: Thomas. 1951.
8. Ishizaka K, Ishizaka T, Menzel AE. Physicochemical properties of reaginic antibody. VI. Effect of heat on gamma-E-, gamma-G- and gamma-A-antibodies in the sera of ragweed sensitive patients. J Immunol. 1967;99(3):610-8.
9. Wide L, Bennich H, Johansson SG. Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet. 1967;2(7526):1105-7.
10. Burks AW, Jones SM, Boyce JA, Sicherer SH, Wood RA, Assa'ad A, et al. NIAID-sponsored 2010 guidelines for managing food allergy: applications in the pediatric population. Pediatrics. 2011;128(5):955-65.
11. Kondo Y, Urisu A. Oral allergy syndrome. Allergol Int. 2009;58(4):485-91.
12. Castillo NE, Theethira TG, Leffler DA. The present and the future in the diagnosis and management of celiac disease. Gastroenterology report. 2014.
13. Jarvinen KM, Nowak-Wegrzyn A. Food protein-induced enterocolitis syndrome (FPIES): current management strategies and review of the literature. The journal of allergy and clinical immunology in practice. 2013;1(4):317-22.
14. Fernandes BN, Boyle RJ, Gore C, Simpson A, Custovic A. Food protein-induced enterocolitis syndrome can occur in adults. J Allergy Clin Immunol. 2012;130(5):1199-200.
15. Franciosi JP, Liacouras CA. Eosinophilic esophagitis. Immunol Allergy Clin North Am. 2009;29(1):19-27, viii.
16. Bischoff SC. Food allergy and eosinophilic gastroenteritis and colitis. Curr Opin Allergy Clin Immunol. 2010;10(3):238-45.
17. Maintz L, Novak N. Histamine and histamine intolerance. The American journal of clinical nutrition. 2007;85(5):1185-96.
18. Gultekin F, Doguc DK. Allergic and immunologic reactions to food additives. Clinical reviews in allergy & immunology. 2013;45(1):6-29.

19. Wilt TJ, Shaukat A, Shamliyan T, Taylor BC, MacDonald R, Tacklind J, et al. Lactose intolerance and health. Evidence report/technology assessment. 2010(192):1-410.
20. Murray RJ. Recognition and management of Staphylococcus aureus toxin-mediated disease. Internal medicine journal. 2005;35 Suppl 2:S106-19.
21. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, et al. ICON: food allergy. J Allergy Clin Immunol. 2012;129(4):906-20.
22. Altman DR, Chiaramonte LT. Public perception of food allergy. J Allergy Clin Immunol. 1996;97(6):1247-51.
23. Schafer T, Bohler E, Ruhdorfer S, Weigl L, Wessner D, Heinrich J, et al. Epidemiology of food allergy/food intolerance in adults: associations with other manifestations of atopy. Allergy. 2001;56(12):1172-9.
24. Woods RK, Abramson M, Bailey M, Walters EH. International prevalences of reported food allergies and intolerances. Comparisons arising from the European Community Respiratory Health Survey (ECRHS) 1991-1994. Eur J Clin Nutr. 2001;55(4):298-304.
25. Chafen JJ, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, et al. Diagnosing and managing common food allergies: a systematic review. JAMA. 2010;303(18):1848-56.
26. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol. 2007;120(3):638-46.
27. Sicherer SH. Epidemiology of food allergy. J Allergy Clin Immunol. 2011;127(3):594-602.
28. Perry TT, Pesek RD. Clinical manifestations of food allergy. Pediatric annals. 2013;42(6):96-101.
29. Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, et al. National prevalence and risk factors for food allergy and relationship to asthma: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol. 2010;126(4):798-806 e13.
30. Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. J Allergy Clin Immunol. 2014;133(2):291-307 e5.
31. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. Allergy. 2014;69(3):365-71.
32. McGowan EC, Keet CA. Prevalence of self-reported food allergy in the National Health and Nutrition Examination Survey (NHANES) 2007-2010. J Allergy Clin Immunol. 2013;132(5):1216-9 e5.
33. Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Lowe AJ, Matheson MC, et al. Prevalence of challenge-proven IgE-mediated food

allergy using population-based sampling and predetermined challenge criteria in infants. J Allergy Clin Immunol. 2011;127(3):668-76 e1-2.
34. Lee AJ, Thalayasingam M, Lee BW. Food allergy in Asia: how does it compare? Asia Pacific allergy. 2013;3(1):3-14.
35. Park M, Kim D, Ahn K, Kim J, Han Y. Prevalence of immediate-type food allergy in early childhood in seoul. Allergy Asthma Immunol Res. 2014;6(2):131-6.
36. Branum AM, Lukacs SL. Food allergy among children in the United States. Pediatrics. 2009;124(6):1549-55.
37. Chaudhry RQ, Oppenheimer JJ. Update on food allergy in adults. Current allergy and asthma reports. 2012;12(4):311-20.
38. McClain S, Bowman C, Fernandez-Rivas M, Ladics GS, Ree R. Allergic sensitization: food- and protein-related factors. Clinical and translational allergy. 2014;4(1):11.
39. Lack G. Update on risk factors for food allergy. J Allergy Clin Immunol. 2012;129(5):1187-97.
40. Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol. 2011;127(5):1195-202.
41. Vuillermin PJ, Ponsonby AL, Kemp AS, Allen KJ. Potential links between the emerging risk factors for food allergy and vitamin D status. Clin Exp Allergy. 2013;43(6):599-607.
42. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. Lancet. 1994;343(8906):1127-30.
43. Abbas AL, AH; Pilli, S. Cellular and molecular immunology. Saunders. 2013.
44. Brandtzaeg P, Bjerke K, Kett K, Kvale D, Rognum TO, Scott H, et al. Production and secretion of immunoglobulins in the gastrointestinal tract. Annals of allergy. 1987;59(5 Pt 2):21-39.
45. Widmaier ER, H; Strang, K. Vander's Human Physiology: The Mechanisms of Body Function 13th edition. McGraw-Hill Science/Engineering/Math. 2013.
46. MacDonald TT. Effector and regulatory lymphoid cells and cytokines in mucosal sites. Current topics in microbiology and immunology. 1999;236:113-35.
47. Berin MC, Sampson HA. Mucosal immunology of food allergy. Current biology : CB. 2013;23(9):R389-400.
48. Metcalfe DDSHASRA. Food Allergy: Adverse reactions to foods and food additives. Blackwell Publishing. 2008;Fourth Edition.
49. Otsu K, Dreskin SC. Peanut allergy: an evolving clinical challenge. Discovery medicine. 2011;12(65):319-28.
50. Webb KE, Jr. Amino acid and peptide absorption from the gastrointestinal tract. Federation proceedings. 1986;45(8):2268-71.

51. Inobe J, Slavin AJ, Komagata Y, Chen Y, Liu L, Weiner HL. IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. Eur J Immunol. 1998;28(9):2780-90.
52. Frossard CP, Tropia L, Hauser C, Eigenmann PA. Lymphocytes in Peyer patches regulate clinical tolerance in a murine model of food allergy. J Allergy Clin Immunol. 2004;113(5):958-64.
53. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299(5609):1057-61.
54. Ramsdell F, Ziegler SF. FOXP3 and scurfy: how it all began. Nat Rev Immunol. 2014;14(5):343-9.
55. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med. 2004;199(12):1679-88.
56. Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. J Immunol. 1994;152(9):4663-70.
57. Kraus TA, Brimnes J, Muong C, Liu JH, Moran TM, Tappenden KA, et al. Induction of mucosal tolerance in Peyer's patch-deficient, ligated small bowel loops. J Clin Invest. 2005;115(8):2234-43.
58. Kunkel EJ, Campbell DJ, Butcher EC. Chemokines in lymphocyte trafficking and intestinal immunity. Microcirculation. 2003;10(3-4):313-23.
59. Man AL, Bertelli E, Regoli M, Chambers SJ, Nicoletti C. Antigen-specific T cell-mediated apoptosis of dendritic cells is impaired in a mouse model of food allergy. J Allergy Clin Immunol. 2004;113(5):965-72.
60. Mowat AM, Donachie AM, Parker LA, Robson NC, Beacock-Sharp H, McIntyre LJ, et al. The role of dendritic cells in regulating mucosal immunity and tolerance. Novartis Foundation symposium. 2003;252:291-302; discussion -5.
61. Karlsson M, Lundin S, Dahlgren U, Kahu H, Pettersson I, Telemo E. "Tolerosomes" are produced by intestinal epithelial cells. Eur J Immunol. 2001;31(10):2892-900.
62. Ostman S, Taube M, Telemo E. Tolerosome-induced oral tolerance is MHC dependent. Immunology. 2005;116(4):464-76.
63. Bengtsson UE, N.E. Förrädisk föda: om överkänslighet för mat och dryck. AstraZeneca, Sweden. 2003.
64. Bohn L, Storsrud S, Tornblom H, Bengtsson U, Simren M. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. Am J Gastroenterol. 2013;108(5):634-41.
65. Simren M, Mansson A, Langkilde AM, Svedlund J, Abrahamsson H, Bengtsson U, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. Digestion. 2001;63(2):108-15.

66. Lillestol K, Helgeland L, Arslan Lied G, Florvaag E, Valeur J, Lind R, et al. Indications of 'atopic bowel' in patients with self-reported food hypersensitivity. Aliment Pharmacol Ther.31(10):1112-22.
67. Magnusson J, Lin XP, Dahlman-Hoglund A, Hanson LL, Telemo E, Magnusson O, et al. Seasonal intestinal inflammation in patients with birch pollen allergy. J Allergy Clin Immunol. 2003;112(1):45-50.
68. Rentzos G, Lundberg V, Stotzer PO, Pullerits T, Telemo E. Intestinal allergic inflammation in birch pollen allergic patients in relation to pollen season, IgE sensitization profile and gastrointestinal symptoms. Clinical and translational allergy. 2014;4:19.
69. Almansa C, Krishna M, Buchner AM, Ghabril MS, Talley N, DeVault KR, et al. Seasonal distribution in newly diagnosed cases of eosinophilic esophagitis in adults. Am J Gastroenterol. 2009;104(4):828-33.
70. Onbasi K, Sin AZ, Doganavsargil B, Onder GF, Bor S, Sebik F. Eosinophil infiltration of the oesophageal mucosa in patients with pollen allergy during the season. Clin Exp Allergy. 2005;35(11):1423-31.
71. Aceves SS. Food allergy testing in eosinophilic esophagitis: what the gastroenterologist needs to know. Clin Gastroenterol Hepatol. 2014;12(8):1216-23.
72. Powe DG, Bonnin AJ, Jones NS. 'Entopy': local allergy paradigm. Clin Exp Allergy. 2010;40(7):987-97.
73. Sabirov A, Hamilton RG, Jacobs JB, Hillman DE, Lebowitz RA, Watts JD. Role of local immunoglobulin E specific for Alternaria alternata in the pathogenesis of nasal polyposis. The Laryngoscope. 2008;118(1):4-9.
74. Amin K, Rinne J, Haahtela T, Simola M, Peterson CG, Roomans GM, et al. Inflammatory cell and epithelial characteristics of perennial allergic and nonallergic rhinitis with a symptom history of 1 to 3 years' duration. J Allergy Clin Immunol. 2001;107(2):249-57.
75. Vagic D, Ferencic Z, Drvis P, Geber G, Dzidic S, Baudoin T, et al. Local IgE and inflammation in chronic rhinosinusitis of asthmatics and non-asthmatics. Eur Arch Otorhinolaryngol. 2008;265(10):1205-9.
76. Beeh KM, Ksoll M, Buhl R. Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. Eur Respir J. 2000;16(4):609-14.
77. Lin XP, Magnusson J, Ahlstedt S, Dahlman-Hoglund A, Hanson LL, Magnusson O, et al. Local allergic reaction in food-hypersensitive adults despite a lack of systemic food-specific IgE. J Allergy Clin Immunol. 2002;109(5):879-87.
78. Bengtsson U, Knutson TW, Knutson L, Dannaeus A, Hallgren R, Ahlstedt S. Eosinophil cationic protein and histamine after intestinal challenge in patients with cow's milk intolerance. J Allergy Clin Immunol. 1997;100(2):216-21.
79. Arslan G, Lillestol K, Mulahasanovic A, Florvaag E, Berstad A. Food hypersensitivity reactions visualised by ultrasonography and magnetic

resonance imaging in a patient lacking systemic food-specific IgE. Digestion. 2006;73(2-3):111-5.
80. Robertson D, Wright R. Oedema of the ileum: a possible manifestation of food allergy. Br Med J (Clin Res Ed). 1987;294(6568):350.
81. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. J Allergy Clin Immunol. 2009;124(1):3-20; quiz 1-2.
82. Perrier C, Corthesy B. Gut permeability and food allergies. Clin Exp Allergy. 2011;41(1):20-8.
83. Benard A, Desreumeaux P, Huglo D, Hoorelbeke A, Tonnel AB, Wallaert B. Increased intestinal permeability in bronchial asthma. J Allergy Clin Immunol. 1996;97(6):1173-8.
84. Buhner S, Reese I, Kuehl F, Lochs H, Zuberbier T. Pseudoallergic reactions in chronic urticaria are associated with altered gastroduodenal permeability. Allergy. 2004;59(10):1118-23.
85. Pike MG, Heddle RJ, Boulton P, Turner MW, Atherton DJ. Increased intestinal permeability in atopic eczema. J Invest Dermatol. 1986;86(2):101-4.
86. Bengtsson U, Knutson TW, Knutson L, Dannaeus A, Hallgren R, Ahlstedt S. Increased levels of hyaluronan and albumin after intestinal challenge in adult patients with cow's milk intolerance. Clin Exp Allergy. 1996;26(1):96-103.
87. Bengtsson U, Hanson LA, Ahlstedt S. Survey of gastrointestinal reactions to foods in adults in relation to atopy, presence of mucus in the stools, swelling of joints and arthralgia in patients with gastrointestinal reactions to foods. Clin Exp Allergy. 1996;26(12):1387-94.
88. Jacobsen MB, Aukrust P, Kittang E, Muller F, Ueland T, Bratlie J, et al. Relation between food provocation and systemic immune activation in patients with food intolerance. Lancet. 2000;356(9227):400-1.
89. Pacor ML, Lunardi C, Di Lorenzo G, Biasi D, Corrocher R. Food allergy and seronegative arthritis: report of two cases. Clin Rheumatol. 2001;20(4):279-81.
90. Panush RS. Does food cause or cure arthritis? Rheumatic diseases clinics of North America. 1991;17(2):259-72.
91. Panush RS, Stroud RM, Webster EM. Food-induced (allergic) arthritis. Inflammatory arthritis exacerbated by milk. Arthritis and rheumatism. 1986;29(2):220-6.
92. Ratner D, Eshel E, Vigder K. Juvenile rheumatoid arthritis and milk allergy. Journal of the Royal Society of Medicine. 1985;78(5):410-3.
93. Schrander JJ, Marcelis C, de Vries MP, van Santen-Hoeufft HM. Does food intolerance play a role in juvenile chronic arthritis? Br J Rheumatol. 1997;36(8):905-8.
94. Williams R. Rheumatoid arthritis and food: a case study. Br Med J (Clin Res Ed). 1981;283(6290):563.

95. Lunardi C, Bambara LM, Biasi D, Venturini G, Nicolis F, Pachor ML, et al. Food allergy and rheumatoid arthritis. Clin Exp Rheumatol. 1988;6(4):423-4.
96. Sampson HA. Food anaphylaxis. British medical bulletin. 2000;56(4):925-35.
97. Jarvinen KM. Food-induced anaphylaxis. Curr Opin Allergy Clin Immunol. 2011;11(3):255-61.
98. Brown SG. Clinical features and severity grading of anaphylaxis. J Allergy Clin Immunol. 2004;114(2):371-6.
99. McKiernan CA, Lieberman SA. Circulatory shock in children: an overview. Pediatrics in review / American Academy of Pediatrics. 2005;26(12):451-60.
100. Pumphrey RS, Gowland MH. Further fatal allergic reactions to food in the United Kingdom, 1999-2006. J Allergy Clin Immunol. 2007;119(4):1018-9.
101. Johnson RF, Peebles RS. Anaphylactic shock: pathophysiology, recognition, and treatment. Seminars in respiratory and critical care medicine. 2004;25(6):695-703.
102. Lieberman P. Biphasic anaphylactic reactions. Ann Allergy Asthma Immunol. 2005;95(3):217-26; quiz 26, 58.
103. Wolanczyk-Medrala A, Barg W, Radlinska A, Panaszek B, Medrala W. Food-dependent exercise-induced anaphylaxis-sequence of causative factors might be reversed. Annals of agricultural and environmental medicine : AAEM. 2010;17(2):315-7.
104. Commins SP, Platts-Mills TA. Delayed anaphylaxis to red meat in patients with IgE specific for galactose alpha-1,3-galactose (alpha-gal). Current allergy and asthma reports. 2013;13(1):72-7.
105. Commins SP, Platts-Mills TA. Tick bites and red meat allergy. Curr Opin Allergy Clin Immunol. 2013;13(4):354-9.
106. Music E, Korosec P, Silar M, Adamic K, Kosnik M, Rijavec M. Serum diamine oxidase activity as a diagnostic test for histamine intolerance. Wien Klin Wochenschr. 2013;125(9-10):239-43.
107. Grundy J, Matthews S, Bateman B, Dean T, Arshad SH. Rising prevalence of allergy to peanut in children: Data from 2 sequential cohorts. J Allergy Clin Immunol. 2002;110(5):784-9.
108. Nicolaou N, Poorafshar M, Murray C, Simpson A, Winell H, Kerry G, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. J Allergy Clin Immunol. 2010;125(1):191-7.e1-13.
109. Roy KM, Roberts MC. Peanut allergy in children: relationships to health-related quality of life, anxiety, and parental stress. Clinical pediatrics. 2011;50(11):1045-51.
110. Hill DJ, Hosking CS, Zhie CY, Leung R, Baratwidjaja K, Iikura Y, et al. The frequency of food allergy in Australia and Asia. Environmental toxicology and pharmacology. 1997;4(1-2):101-10.

111. Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW, et al. Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol. 2001;107(6):1077-81.
112. Sicherer SH, Sampson HA. 9. Food allergy. J Allergy Clin Immunol. 2006;117(2 Suppl Mini-Primer):S470-5.
113. Sicherer SH, Wood RA, American Academy of Pediatrics Section On A, Immunology. Allergy testing in childhood: using allergen-specific IgE tests. Pediatrics. 2012;129(1):193-7.
114. Roberts G, Lack G. Diagnosing peanut allergy with skin prick and specific IgE testing. J Allergy Clin Immunol. 2005;115(6):1291-6.
115. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. Clin Exp Allergy. 2000;30(11):1540-6.
116. Masthoff LJ, van Hoffen E, de Reus A, Boonacker CW, Bruijnzeel-Koomen CA, Pasmans SG, et al. Hazelnut allergy differs between children and adults in frequency of severity, aetiology and relevance of diagnostic parameters. Clin Exp Allergy. 2014;44(12):1539-45.
117. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. Allergy. 2009;64(10):1498-506.
118. Burbach GJ, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. Allergy. 2009;64(10):1507-15.
119. Bernstein IL, Storms WW. Practice parameters for allergy diagnostic testing. Joint Task Force on Practice Parameters for the Diagnosis and Treatment of Asthma. The American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol. 1995;75(6 Pt 2):543-625.
120. Dreborg S. The skin prick test in the diagnosis of atopic allergy. Journal of the American Academy of Dermatology. 1989;21(4 Pt 2):820-1.
121. Levy SA, Dortas Junior SD, Pires AH, Abe AT, Valle SO, Coelho VP, et al. Atopy patch test (APT) in the diagnosis of food allergy in children with atopic dermatitis. Anais brasileiros de dermatologia. 2012;87(5):724-8.
122. Mowszet K, Matusiewicz K, Iwanczak B. Value of the atopy patch test in the diagnosis of food allergy in children with gastrointestinal symptoms. Advances in clinical and experimental medicine : official organ Wroclaw Medical University. 2014;23(3):403-9.
123. Celakovska J, Vaneckova J, Ettlerova K, Ettler K, Bukac J. The role of atopy patch test in diagnosis of food allergy in atopic eczema/dermatitis syndrom in patients over 14 years of age. Acta medica (Hradec Kralove) / Universitas Carolina, Facultas Medica Hradec Kralove. 2010;53(2):101-8.

124. Hamilton RG, Williams PB. Human IgE antibody serology: a primer for the practicing North American allergist/immunologist. J Allergy Clin Immunol. 2010;126(1):33-8.
125. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol. 2001;107(5):891-6.
126. Benhamou AH, Zamora SA, Eigenmann PA. Correlation between specific immunoglobulin E levels and the severity of reactions in egg allergic patients. Pediatr Allergy Immunol. 2008;19(2):173-9.
127. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy. 2005;35(3):268-73.
128. Lock RJ, Unsworth DJ. Food allergy: which tests are worth doing and which are not? Annals of clinical biochemistry. 2011;48(Pt 4):300-9.
129. Ortolani C, Ispano M, Pastorello EA, Ansaloni R, Magri GC. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. J Allergy Clin Immunol. 1989;83(3):683-90.
130. Sharnan J, Kumar L, Singh S. Comparison of results of skin prick tests, enzyme-linked immunosorbent assays and food challenges in children with respiratory allergy. J Trop Pediatr. 2001;47(6):367-8.
131. Schoos AM, Chawes BL, Folsgaard NV, Samandari N, Bonnelykke K, Bisgaard H. Disagreement between skin prick test and specific IgE in young children. Allergy. 2015;70(1):41-8.
132. Nettis E, Bonifazi F, Bonini S, Di Leo E, Maggi E, Melioli G, et al. Molecular diagnosis and the Italian Board for ISAC. Eur Ann Allergy Clin Immunol. 2014;46(2):68-73.
133. Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Gronlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy. 1999;29(7):896-904.
134. Melioli G, Bonifazi F, Bonini S, Maggi E, Mussap M, Passalacqua G, et al. The ImmunoCAP ISAC molecular allergology approach in adult multi-sensitized Italian patients with respiratory symptoms. Clin Biochem. 2011;44(12):1005-11.
135. Harwanegg C, Hiller R. Protein microarrays in diagnosing IgE-mediated diseases: spotting allergy at the molecular level. Expert review of molecular diagnostics. 2004;4(4):539-48.
136. Sastre J. Molecular diagnosis in allergy. Clin Exp Allergy. 2010;40(10):1442-60.
137. www.thermoscientific.com.
138. Melioli G, Canonica GW. Molecular allergy diagnosis: we need to become more knowledgeable. Ann Allergy Asthma Immunol. 2012;108(6):387.

139. Asero R, Villalta D. Profilin may be a primary airborne sensitizer: a case report. J Investig Allergol Clin Immunol. 2013;23(2):134-5.
140. Asero R. Component-resolved diagnosis-assisted prescription of allergen-specific immunotherapy: a practical guide. Eur Ann Allergy Clin Immunol. 2012;44(5):183-7.
141. Passalacqua G, Melioli G, Bonifazi F, Bonini S, Maggi E, Senna G, et al. The additional values of microarray allergen assay in the management of polysensitized patients with respiratory allergy. Allergy. 2013;68(8):1029-33.
142. Sebei K, Gnouma A, Herchi W, Sakouhi F, Boukhchina S. Lipids, proteins, phenolic composition, antioxidant and antibacterial activities of seeds of peanuts (Arachis hypogaea l) cultivated in Tunisia. Biological research. 2013;46(3):257-63.
143. Koppelman SJ, Vlooswijk RA, Knippels LM, Hessing M, Knol EF, van Reijsen FC, et al. Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. Allergy. 2001;56(2):132-7.
144. Hebling CM, Ross MM, Callahan JH, McFarland MA. Size-selective fractionation and visual mapping of allergen protein chemistry in Arachis hypogaea. Journal of proteome research. 2012;11(11):5384-95.
145. Ebisawa M, Moverare R, Sato S, Maruyama N, Borres MP, Komata T. Measurement of Ara h 1-, 2-, and 3-specific IgE antibodies is useful in diagnosis of peanut allergy in Japanese children. Pediatr Allergy Immunol. 2012;23(6):573-81.
146. Klemans RJ, Otte D, Knol M, Knol EF, Meijer Y, Gmelig-Meyling FH, et al. The diagnostic value of specific IgE to Ara h 2 to predict peanut allergy in children is comparable to a validated and updated diagnostic prediction model. J Allergy Clin Immunol. 2013;131(1):157-63.
147. Flinterman AE, van Hoffen E, den Hartog Jager CF, Koppelman S, Pasmans SG, Hoekstra MO, et al. Children with peanut allergy recognize predominantly Ara h2 and Ara h6, which remains stable over time. Clin Exp Allergy. 2007;37(8):1221-8.
148. Mittag D, Akkerdaas J, Ballmer-Weber BK, Vogel L, Wensing M, Becker WM, et al. Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. J Allergy Clin Immunol. 2004;114(6):1410-7.
149. Klemans RJ, Broekman HC, Knol EF, Bruijnzeel-Koomen CA, Otten HG, Pasmans SG, et al. Ara h 2 is the best predictor for peanut allergy in adults. The journal of allergy and clinical immunology in practice. 2013;1(6):632-8.e1.
150. Lauer I, Dueringer N, Pokoj S, Rehm S, Zoccatelli G, Reese G, et al. The non-specific lipid transfer protein, Ara h 9, is an important allergen in peanut. Clin Exp Allergy. 2009;39(9):1427-37.

151. Peeters KA, Koppelman SJ, Penninks AH, Lebens A, Bruijnzeel-Koomen CA, Hefle SL, et al. Clinical relevance of sensitization to lupine in peanut-sensitized adults. Allergy. 2009;64(4):549-55.
152. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. J Allergy Clin Immunol. 2003;112(6):1203-7.
153. Clark AT, Ewan PW. The development and progression of allergy to multiple nuts at different ages. Pediatr Allergy Immunol. 2005;16(6):507-11.
154. Maloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. J Allergy Clin Immunol. 2008;122(1):145-51.
155. Glaspole IN, de Leon MP, Prickett SR, O'Hehir RE, Rolland JM. Clinical allergy to hazelnut and peanut: identification of T cell cross-reactive allergens. Int Arch Allergy Immunol. 2011;155(4):345-54.
156. Eller E, Bindslev-Jensen C. Clinical value of component-resolved diagnostics in peanut-allergic patients. Allergy. 2013;68(2):190-4.
157. Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. Allergy. 2015;70(1):90-8.
158. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods--position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004;59(7):690-7.
159. Gellerstedt M, Bengtsson U, Niggemann B. Methodological issues in the diagnostic work-up of food allergy: a real challenge. J Investig Allergol Clin Immunol. 2007;17(6):350-6.
160. Gellerstedt M, Magnusson J, Grajo U, Ahlstedt S, Bengtsson U. Interpretation of subjective symptoms in double-blind placebo-controlled food challenges - interobserver reliability. Allergy. 2004;59(3):354-6.
161. Klein Entink RH, Remington BC, Blom WM, Rubingh CM, Kruizinga AG, Baumert JL, et al. Food allergy population thresholds: an evaluation of the number of oral food challenges and dosing schemes on the accuracy of threshold dose distribution modeling. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2014;70:134-43.
162. Grimshaw KE, King RM, Nordlee JA, Hefle SL, Warner JO, Hourihane JO. Presentation of allergen in different food preparations affects the nature of the allergic reaction--a case series. Clin Exp Allergy. 2003;33(11):1581-5.
163. van Odijk J, Ahlstedt S, Bengtsson U, Borres MP, Hulthen L. Double-blind placebo-controlled challenges for peanut allergy the efficiency

of blinding procedures and the allergenic activity of peanut availability in the recipes. Allergy. 2005;60(5):602-5.
164. Togias AG, Naclerio RM, Warner J, Proud D, Kagey-Sobotka A, Nimmagadda I, et al. Demonstration of inhibition of mediator release from human mast cells by azatadine base. In vivo and in vitro evaluation. Jama. 1986;255(2):225-9.
165. Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. J Allergy Clin Immunol. 1991;88(3 Pt 1):328-38.
166. McGowan EC, Saini S. Update on the performance and application of basophil activation tests. Current allergy and asthma reports. 2013;13(1):101-9.
167. MacGlashan DW, Jr. Basophil activation testing. J Allergy Clin Immunol. 2013;132(4):777-87.
168. Crivellato E, Nico B, Mallardi F, Beltrami CA, Ribatti D. Piecemeal degranulation as a general secretory mechanism? The anatomical record Part A, Discoveries in molecular, cellular, and evolutionary biology. 2003;274(1):778-84.
169. Furuno T, Teshima R, Kitani S, Sawada J, Nakanishi M. Surface expression of CD63 antigen (AD1 antigen) in P815 mastocytoma cells by transfected IgE receptors. Biochem Biophys Res Commun. 1996;219(3):740-4.
170. MacGlashan D, Jr. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. Clin Exp Allergy. 2010;40(9):1365-77.
171. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: technical issues and critical factors. Allergy. 2009;64(9):1319-26.
172. Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: present and future applications in allergology. Cytometry B Clin Cytom. 2008;74(4):201-10.
173. Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, De Clerck LS, Stevens WJ. Comments regarding 'Marked improvement of the basophil activation test by detecting CD203c instead of CD63' by Boumiza et al. Clin Exp Allergy. 2003;33(6):849; author reply 52-3.
174. Gyimesi E, Sipka S, Danko K, Kiss E, Hidvegi B, Gal M, et al. Basophil CD63 expression assay on highly sensitized atopic donor leucocytes-a useful method in chronic autoimmune urticaria. Br J Dermatol. 2004;151(2):388-96.
175. Erdmann SM, Sachs B, Schmidt A, Merk HF, Scheiner O, Moll-Slodowy S, et al. In vitro analysis of birch-pollen-associated food allergy by use of recombinant allergens in the basophil activation test. Int Arch Allergy Immunol. 2005;136(3):230-8.
176. Kahlert H, Cromwell O, Fiebig H. Measurement of basophil-activating capacity of grass pollen allergens, allergoids and hypoallergenic

recombinant derivatives by flow cytometry using anti-CD203c. Clin Exp Allergy. 2003;33(9):1266-72.
177. Sanz ML, Garcia-Aviles MC, Tabar AI, Anda M, Garcia BE, Barber D, et al. Basophil Activation Test and specific IgE measurements using a panel of recombinant natural rubber latex allergens to determine the latex allergen sensitization profile in children. Pediatr Allergy Immunol. 2006;17(2):148-56.
178. Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. Ann Allergy Asthma Immunol. 1999;82(1):33-40.
179. Aranda A, Mayorga C, Ariza A, Dona I, Rosado A, Blanca-Lopez N, et al. In vitro evaluation of IgE-mediated hypersensitivity reactions to quinolones. Allergy. 2011;66(2):247-54.
180. De Week AL, Sanz ML, Gamboa PM, Aberer W, Sturm G, Bilo MB, et al. Diagnosis of immediate-type beta-lactam allergy in vitro by flow-cytometric basophil activation test and sulfidoleukotriene production: a multicenter study. J Investig Allergol Clin Immunol. 2009;19(2):91-109.
181. Eberlein B, Leon Suarez I, Darsow U, Rueff F, Behrendt H, Ring J. A new basophil activation test using CD63 and CCR3 in allergy to antibiotics. Clin Exp Allergy. 2010;40(3):411-8.
182. Gamboa P, Sanz ML, Caballero MR, Urrutia I, Antepara I, Esparza R, et al. The flow-cytometric determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. Clin Exp Allergy. 2004;34(9):1448-57.
183. Kim MS, Cho YJ. Flow Cytometry-Assisted Basophil Activation Test as a Safe Diagnostic Tool for Aspirin/NSAID Hypersenstivity. Allergy Asthma Immunol Res. 2012;4(3):137-42.
184. Rodriguez-Trabado A, Camara-Hijon C, Ramos-Cantarino A, Porcel-Carreno SL, Jimenez-Timon S, Pereira-Navarro G, et al. Basophil activation test for the in vitro diagnosis of nonsteroidal anti-inflammatory drug hypersensitivity. Allergy Asthma Proc. 2008;29(3):241-9.
185. Pinnobphun P, Buranapraditkun S, Kampitak T, Hirankarn N, Klaewsongkram J. The diagnostic value of basophil activation test in patients with an immediate hypersensitivity reaction to radiocontrast media. Ann Allergy Asthma Immunol. 2011;106(5):387-93.
186. Hagau N, Gherman-Ionica N, Sfichi M, Petrisor C. Threshold for basophil activation test positivity in neuromuscular blocking agents hypersensitivity reactions. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology. 2013;9(1):42.
187. Leysen J, Sabato V, Verweij MM, De Knop KJ, Bridts CH, De Clerck LS, et al. The basophil activation test in the diagnosis of immediate drug hypersensitivity. Expert Rev Clin Immunol. 2011;7(3):349-55.

188. Nopp A, Cardell LO, Johansson SG. CD-sens can be a reliable and easy-to-use complement in the diagnosis of allergic rhinitis. Int Arch Allergy Immunol. 2013;161(1):87-90.
189. Ozdemir SK, Guloglu D, Sin BA, Elhan AH, Ikinciogullari A, Misirligil Z. Reliability of basophil activation test using CD203c expression in diagnosis of pollen allergy. American journal of rhinology & allergy. 2011;25(6):e225-31.
190. Wolanczyk-Medrala A, Gogolewski G, Liebhart J, Gomulka K, Litwa M, Panaszek B, et al. A new variant of the basophil activation test for allergen-induced basophil CD63 upregulation. The effect of cetirizine. J Investig Allergol Clin Immunol. 2009;19(6):465-73.
191. Eberlein B. Basophil activation test in the diagnosis of insect venom allergies. Clin Exp Allergy. 2009;39(11):1633-4.
192. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. Double positivity to bee and wasp venom: improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. J Allergy Clin Immunol. 2012;130(1):155-61.
193. Kosnik M, Korosec P. Importance of basophil activation testing in insect venom allergy. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology. 2009;5(1):11.
194. Peternelj A, Silar M, Bajrovic N, Adamic K, Music E, Kosnik M, et al. Diagnostic value of the basophil activation test in evaluating Hymenoptera venom sensitization. Wien Klin Wochenschr. 2009;121(9-10):344-8.
195. Nopp A, Cardell LO, Johansson SG, Oman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. Allergy. 2009;64(5):811-4.
196. Zitnik SE, Vesel T, Avcin T, Silar M, Kosnik M, Korosec P. Monitoring honeybee venom immunotherapy in children with the basophil activation test. Pediatr Allergy Immunol. 2012;23(2):166-72.
197. Sato S, Tachimoto H, Shukuya A, Kurosaka N, Yanagida N, Utsunomiya T, et al. Basophil activation marker CD203c is useful in the diagnosis of hen's egg and cow's milk allergies in children. Int Arch Allergy Immunol. 2010;152 Suppl 1:54-61.
198. Ocmant A, Mulier S, Hanssens L, Goldman M, Casimir G, Mascart F, et al. Basophil activation tests for the diagnosis of food allergy in children. Clin Exp Allergy. 2009;39(8):1234-45.
199. Tokuda R, Nagao M, Hiraguchi Y, Hosoki K, Matsuda T, Kouno K, et al. Antigen-induced expression of CD203c on basophils predicts IgE-mediated wheat allergy. Allergol Int. 2009;58(2):193-9.
200. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. Flow cytometric analysis of in vitro activated

basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. Cytometry B Clin Cytom. 2005;64(1):28-33.
201. Kim JH, An S, Kim JE, Choi GS, Ye YM, Park HS. Beef-induced anaphylaxis confirmed by the basophil activation test. Allergy Asthma Immunol Res. 2010;2(3):206-8.
202. Wallowitz ML, Chen RJ, Tzen JT, Teuber SS. Ses i 6, the sesame 11S globulin, can activate basophils and shows cross-reactivity with walnut in vitro. Clin Exp Allergy. 2007;37(6):929-38.
203. Carroccio A, Brusca I, Mansueto P, D'Alcamo A, Barrale M, Soresi M, et al. A comparison between two different in vitro basophil activation tests for gluten- and cow's milk protein sensitivity in irritable bowel syndrome (IBS)-like patients. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2013;51(6):1257-63.
204. Rubio A, Vivinus-Nebot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. Allergy. 2011;66(1):92-100.
205. Vila L, Moreno A, Gamboa PM, Martinez-Aranguren R, Sanz ML. Decrease in antigen-specific CD63 basophil expression is associated with the development of tolerance to egg by SOTI in children. Pediatr Allergy Immunol. 2013;24(5):463-8.
206. Sabato V, van Hengel AJ, De Knop KJ, Verweij MM, Hagendorens MM, Bridts CH, et al. Basophil activation reveals divergent patient-specific responses to thermally processed peanuts. J Investig Allergol Clin Immunol. 2011;21(7):527-31.
207. Glaumann S, Nopp A, Johansson SG, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. Allergy. 2012;67(2):242-7.
208. Santos AF, Douiri A, Becares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin Immunol. 2014;134(3):645-52.
209. Hogan SP, Rothenberg ME. Review article: The eosinophil as a therapeutic target in gastrointestinal disease. Aliment Pharmacol Ther. 2004;20(11-12):1231-40.
210. Bischoff S, Crowe SE. Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives. Gastroenterology. 2005;128(4):1089-113.
211. Bischoff SC, Ulmer FA. Eosinophils and allergic diseases of the gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2008;22(3):455-79.
212. Rokaite R, Labanauskas L. Gastrointestinal disorders in children with atopic dermatitis. Medicina (Kaunas, Lithuania). 2005;41(10):837-45.

213. Guajardo JR, Plotnick LM, Fende JM, Collins MH, Putnam PE, Rothenberg ME. Eosinophil-associated gastrointestinal disorders: a world-wide-web based registry. J Pediatr. 2002;141(4):576-81.
214. Host A. Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol. 2002;89(6 Suppl 1):33-7.
215. Bischoff SC, Mayer J, Wedemeyer J, Meier PN, Zeck-Kapp G, Wedi B, et al. Colonoscopic allergen provocation (COLAP): a new diagnostic approach for gastrointestinal food allergy. Gut. 1997;40(6):745-53.
216. Hagel AF, de Rossi TM, Zopf Y, Lindner AS, Dauth W, Neurath MF, et al. Small-bowel capsule endoscopy in patients with gastrointestinal food allergy. Allergy. 2012;67(2):286-92.
217. Kristjansson G, Venge P, Hallgren R. Mucosal reactivity to cow's milk protein in coeliac disease. Clin Exp Immunol. 2007;147(3):449-55.
218. Liden M, Kristjansson G, Valtysdottir S, Venge P, Hallgren R. Cow's milk protein sensitivity assessed by the mucosal patch technique is related to irritable bowel syndrome in patients with primary Sjogren's syndrome. Clin Exp Allergy. 2008;38(6):929-35.
219. Liden M, Kristjansson G, Valtysdottir S, Venge P, Hallgren R. Self-reported food intolerance and mucosal reactivity after rectal food protein challenge in patients with rheumatoid arthritis. Scandinavian journal of rheumatology. 2010;39(4):292-8.
220. Doyle LA, Sepehr GJ, Hamilton MJ, Akin C, Castells MC, Hornick JL. A clinicopathologic study of 24 cases of systemic mastocytosis involving the gastrointestinal tract and assessment of mucosal mast cell density in irritable bowel syndrome and asymptomatic patients. Am J Surg Pathol. 2014;38(6):832-43.
221. Mastocytosis ECNo. ECNM Meeting in Odense 4-6 September 2014.
222. Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol. 2011;127(3):616-22.e1.
223. Jahn-Schmid B, Radakovics A, Luttkopf D, Scheurer S, Vieths S, Ebner C, et al. Bet v 1142-156 is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1-related food allergens. J Allergy Clin Immunol. 2005;116(1):213-9.
224. Bohle B, Zwolfer B, Heratizadeh A, Jahn-Schmid B, Antonia YD, Alter M, et al. Cooking birch pollen-related food: divergent consequences for IgE- and T cell-mediated reactivity in vitro and in vivo. J Allergy Clin Immunol. 2006;118(1):242-9.
225. Radauer C, Willerroider M, Fuchs H, Hoffmann-Sommergruber K, Thalhamer J, Ferreira F, et al. Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. Clin Exp Allergy. 2006;36(7):920-9.

226. Alvarado MI, Jimeno L, De La Torre F, Boissy P, Rivas B, Lazaro MJ, et al. Profilin as a severe food allergen in allergic patients overexposed to grass pollen. Allergy. 2014;69(12):1610-6.
227. Karamloo F, Wangorsch A, Kasahara H, Davin LB, Haustein D, Lewis NG, et al. Phenylcoumaran benzylic ether and isoflavonoid reductases are a new class of cross-reactive allergens in birch pollen, fruits and vegetables. European journal of biochemistry / FEBS. 2001;268(20):5310-20.
228. Kelso JM, Jones RT, Tellez R, Yunginger JW. Oral allergy syndrome successfully treated with pollen immunotherapy. Ann Allergy Asthma Immunol. 1995;74(5):391-6.
229. Modrzynski M, Zawisza E, Rapiejko P, Przybylski G. [Specific-pollen immunotherapy in the treatment of oral allergy syndrome in patients with tree pollen hypersensitivity]. Przeglad lekarski. 2002;59(12):1007-10.
230. Czarnecka-Operacz M, Jenerowicz D, Silny W. Oral allergy syndrome in patients with airborne pollen allergy treated with specific immunotherapy. Acta dermatovenerologica Croatica : ADC. 2008;16(1):19-24.
231. Mauro M, Russello M, Incorvaia C, Gazzola G, Frati F, Moingeon P, et al. Birch-apple syndrome treated with birch pollen immunotherapy. Int Arch Allergy Immunol. 2011;156(4):416-22.
232. Okamoto Y, Kurihara K. [A case of oral allergy syndrome whose symptoms were dramatically improved after rush subcutaneous injection immunotherapy with pollen extracts of birch]. Arerugi = [Allergy]. 2012;61(5):652-8.
233. Bird JA, Burks AW. Food allergy and asthma. Primary care respiratory journal : journal of the General Practice Airways Group. 2009;18(4):258-65.
234. Pumphrey RS. Lessons for management of anaphylaxis from a study of fatal reactions. Clin Exp Allergy. 2000;30(8):1144-50.
235. Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, et al. National surveillance for asthma--United States, 1980-2004. MMWR Surveill Summ. 2007;56(8):1-54.
236. Sampson HA, Aceves S, Bock SA, James J, Jones S, Lang D, et al. Food allergy: A practice parameter update-2014. J Allergy Clin Immunol. 2014;134(5):1016-25.e43.
237. Wang J, Sampson HA. Food allergy: recent advances in pathophysiology and treatment. Allergy Asthma Immunol Res. 2009;1(1):19-29.
238. Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D, et al. Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. Allergy. 1995;50(8):623-35.

239. Kanny G, Moneret-Vautrin DA, Flabbee J, Beaudouin E, Morisset M, Thevenin F. Population study of food allergy in France. J Allergy Clin Immunol. 2001;108(1):133-40.
240. Baena-Cagnani CE, Teijeiro A. Role of food allergy in asthma in childhood. Curr Opin Allergy Clin Immunol. 2001;1(2):145-9.
241. Roberts G, Lack G. Food allergy and asthma--what is the link? Paediatric respiratory reviews. 2003;4(3):205-12.
242. Oehling A, Baena Cagnani CE. Food allergy and child asthma. Allergol Immunopathol (Madr). 1980;8(1):7-14.
243. Anto JM, Sunyer J, Rodriguez-Roisin R, Suarez-Cervera M, Vazquez L. Community outbreaks of asthma associated with inhalation of soybean dust. Toxicoepidemiological Committee. N Engl J Med. 1989;320(17):1097-102.
244. Sicherer SH, Furlong TJ, DeSimone J, Sampson HA. Self-reported allergic reactions to peanut on commercial airliners. J Allergy Clin Immunol. 1999;104(1):186-9.
245. Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med. 1992;327(6):380-4.
246. Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. J Pediatr. 1988;113(3):447-51.
247. Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. J Pediatr. 1990;117(4):561-7.
248. Bock SA. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. Pediatrics. 1987;79(5):683-8.
249. Woods RK, Thien F, Raven J, Walters EH, Abramson M. Prevalence of food allergies in young adults and their relationship to asthma, nasal allergies, and eczema. Ann Allergy Asthma Immunol. 2002;88(2):183-9.
250. Bush RK, Taylor SL, Holden K, Nordlee JA, Busse WW. Prevalence of sensitivity to sulfiting agents in asthmatic patients. Am J Med. 1986;81(5):816-20.
251. van der Brempt X, Ledent C, Mairesse M. Rhinitis and asthma caused by occupational exposure to carob bean flour. J Allergy Clin Immunol. 1992;90(6 Pt 1):1008-10.
252. Rossi GL, Corsico A, Moscato G. Occupational asthma caused by milk proteins: report on a case. J Allergy Clin Immunol. 1994;93(4):799-801.
253. James JM, Crespo JF. Allergic reactions to foods by inhalation. Current allergy and asthma reports. 2007;7(3):167-74.

254. Casper R, Zacharisen MC, Fink JN. Occupational asthma secondary to enzymes used in cheese production. Allergy Asthma Proc. 2008;29(4):376-9.
255. Blanco Carmona JG, Juste Picon S, Garces Sotillos M, Rodriguez Gaston P. Occupational asthma in the confectionary industry caused by sensitivity to egg. Allergy. 1992;47(2 Pt 2):190-1.
256. Vogel NM, Katz HT, Lopez R, Lang DM. Food allergy is associated with potentially fatal childhood asthma. J Asthma. 2008;45(10):862-6.
257. Simpson AB, Glutting J, Yousef E. Food allergy and asthma morbidity in children. Pediatric pulmonology. 2007;42(6):489-95.
258. Roberts G, Patel N, Levi-Schaffer F, Habibi P, Lack G. Food allergy as a risk factor for life-threatening asthma in childhood: a case-controlled study. J Allergy Clin Immunol. 2003;112(1):168-74.
259. Berns SH, Halm EA, Sampson HA, Sicherer SH, Busse PJ, Wisnivesky JP. Food allergy as a risk factor for asthma morbidity in adults. J Asthma. 2007;44(5):377-81.
260. Patelis A, Gunnbjornsdottir M, Malinovschi A, Matsson P, Onell A, Hogman M, et al. Population-based study of multiplexed IgE sensitization in relation to asthma, exhaled nitric oxide, and bronchial responsiveness. J Allergy Clin Immunol. 2012;130(2):397-402.e2.
261. Caffarelli C, Deriu FM, Terzi V, Perrone F, De Angelis G, Atherton DJ. Gastrointestinal symptoms in patients with asthma. Arch Dis Child. 2000;82(2):131-5.
262. Powell N, Huntley B, Beech T, Knight W. Upper gastrointestinal symptoms and asthma: a manifestation of allergy? Gut. 2008;57(7):1026-7.
263. Powell N, Huntley B, Beech T, Knight W, Knight H, Corrigan CJ. Increased prevalence of gastrointestinal symptoms in patients with allergic disease. Postgrad Med J. 2007;83(977):182-6.
264. Louis E, Louis R, Drion V, Bonnet V, Lamproye A, Radermecker M, et al. Increased frequency of bronchial hyperresponsiveness in patients with inflammatory bowel disease. Allergy. 1995;50(9):729-33.
265. White AM, Stevens WH, Upton AR, O'Byrne PM, Collins SM. Airway responsiveness to inhaled methacholine in patients with irritable bowel syndrome. Gastroenterology. 1991;100(1):68-74.
266. Wallaert B, Desreumaux P, Copin MC, Tillie I, Benard A, Colombel JF, et al. Immunoreactivity for interleukin 3 and 5 and granulocyte/macrophage colony-stimulating factor of intestinal mucosa in bronchial asthma. J Exp Med. 1995;182(6):1897-904.
267. Zealley AK, Aitken RC, Rosenthal SV. Personality and bronchial asthma. Proceedings of the Royal Society of Medicine. 1971;64(8):825-9.
268. Primeau MN, Kagan R, Joseph L, Lim H, Dufresne C, Duffy C, et al. The psychological burden of peanut allergy as perceived by adults with

peanut allergy and the parents of peanut-allergic children. Clin Exp Allergy. 2000;30(8):1135-43.
269. Bollinger ME, Dahlquist LM, Mudd K, Sonntag C, Dillinger L, McKenna K. The impact of food allergy on the daily activities of children and their families. Ann Allergy Asthma Immunol. 2006;96(3):415-21.
270. Avery NJ, King RM, Knight S, Hourihane JO. Assessment of quality of life in children with peanut allergy. Pediatr Allergy Immunol. 2003;14(5):378-82.
271. Lucarelli S, Frediani T, Zingoni AM, Ferruzzi F, Giardini O, Quintieri F, et al. Food allergy and infantile autism. Panminerva medica. 1995;37(3):137-41.
272. Bidet B, Leboyer M, Descours B, Bouvard MP, Benveniste J. Allergic sensitization in infantile autism. Journal of autism and developmental disorders. 1993;23(2):419-20.
273. Ashwood P, Anthony A, Torrente F, Wakefield AJ. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. Journal of clinical immunology. 2004;24(6):664-73.
274. Jyonouchi H, Geng L, Ruby A, Zimmerman-Bier B. Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. Neuropsychobiology. 2005;51(2):77-85.
275. Dohan FC. Cereals and schizophrenia data and hypothesis. Acta psychiatrica Scandinavica. 1966;42(2):125-52.
276. Zioudrou C, Streaty RA, Klee WA. Opioid peptides derived from food proteins. The exorphins. J Biol Chem. 1979;254(7):2446-9.
277. Addolorato G, Stefanini GF, Capristo E, Caputo F, Gasbarrini A, Gasbarrini G. Anxiety and depression in adult untreated celiac subjects and in patients affected by inflammatory bowel disease: a personality "trait" or a reactive illness? Hepato-gastroenterology. 1996;43(12):1513-7.
278. Pynnonen PA, Isometsa ET, Aronen ET, Verkasalo MA, Savilahti E, Aalberg VA. Mental disorders in adolescents with celiac disease. Psychosomatics. 2004;45(4):325-35.
279. Pynnonen PA, Isometsa ET, Verkasalo MA, Kahkonen SA, Sipila I, Savilahti E, et al. Gluten-free diet may alleviate depressive and behavioural symptoms in adolescents with coeliac disease: a prospective follow-up case-series study. BMC psychiatry. 2005;5:14.
280. Bell IR, Schwartz GE, Amend D, Peterson JM, Stini WA. Sensitization to early life stress and response to chemical odors in older adults. Biological psychiatry. 1994;35(11):857-63.
281. Ross GH. Clinical characteristics of chemical sensitivity: an illustrative case history of asthma and MCS. Environ Health Perspect. 1997;105 Suppl 2:437-41.

282. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. Nutr Res. 2011;31(1):61-75.
283. Dupont C, Kalach N, Soulaines P, Legoue-Morillon S, Piloquet H, Benhamou PH. Cow's milk epicutaneous immunotherapy in children: a pilot trial of safety, acceptability, and impact on allergic reactivity. J Allergy Clin Immunol. 2010;125(5):1165-7.
284. Henson M, Burks AW. The future of food allergy therapeutics. Seminars in immunopathology. 2012;34(5):703-14.
285. Patil SP, Wang J, Song Y, Noone S, Yang N, Wallenstein S, et al. Clinical safety of Food Allergy Herbal Formula-2 (FAHF-2) and inhibitory effect on basophils from patients with food allergy: Extended phase I study. J Allergy Clin Immunol. 2011;128(6):1259-65.e2.
286. Sampson HA, Leung DY, Burks AW, Lack G, Bahna SL, Jones SM, et al. A phase II, randomized, doubleblind, parallelgroup, placebocontrolled oral food challenge trial of Xolair (omalizumab) in peanut allergy. J Allergy Clin Immunol. 2011;127(5):1309-10.e1.
287. Nowak-Wegrzyn A. Future therapies for food allergy. Przeglad lekarski. 2013;70(12):1065-70.
288. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011;127(3):773-86.e1-7.
289. Forsberg A, Abrahamsson TR, Bjorksten B, Jenmalm MC. Pre- and post-natal Lactobacillus reuteri supplementation decreases allergen responsiveness in infancy. Clin Exp Allergy. 2013;43(4):434-42.
290. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet. 2003;361(9372):1869-71.
291. Frossard CP, Steidler L, Eigenmann PA. Oral administration of an IL-10-secreting Lactococcus lactis strain prevents food-induced IgE sensitization. J Allergy Clin Immunol. 2007;119(4):952-9.
292. Schiavi E, Barletta B, Butteroni C, Corinti S, Boirivant M, Di Felice G. Oral therapeutic administration of a probiotic mixture suppresses established Th2 responses and systemic anaphylaxis in a murine model of food allergy. Allergy. 2011;66(4):499-508.
293. Bashir ME, Andersen P, Fuss IJ, Shi HN, Nagler-Anderson C. An enteric helminth infection protects against an allergic response to dietary antigen. J Immunol. 2002;169(6):3284-92.
294. Ekerljung L. Asthma in West Sweden: A translational study from epidemiology to proteomics. Sahlgrenska Academy at University of Gothenburg. 2012.
295. Lundback B, Nystrom L, Rosenhall L, Stjernberg N. Obstructive lung disease in northern Sweden: respiratory symptoms assessed in a postal survey. Eur Respir J. 1991;4(3):257-66.

296. Jarvis D, Newson R, Lotvall J, Hastan D, Tomassen P, Keil T, et al. Asthma in adults and its association with chronic rhinosinusitis: the GA2LEN survey in Europe. Allergy. 2012;67(1):91-8.
297. Ronmark EP, Ekerljung L, Lotvall J, Toren K, Ronmark E, Lundback B. Large scale questionnaire survey on respiratory health in Sweden: effects of late- and non-response. Respir Med. 2009;103(12):1807-15.
298. Dahlman-Hoglund A, Ahlstedt S, Hanson LA, Dahlgren U, Telemo E. Different expression of IL-2 receptor alpha-chain on a lamina propria T cell population and goblet cells in rats orally tolerized or sensitized to ovalbumin (OA) after colonization with an OA-producing Escherichia coli. Clin Exp Immunol. 1996;106(3):534-40.
299. Andrew SM, Jasani B. An improved method for the inhibition of endogenous peroxidase non-deleterious to lymphocyte surface markers. Application to immunoperoxidase studies on eosinophil-rich tissue preparations. Histochem J. 1987;19(8):426-30.
300. Skypala IJ, Calderon MA, Leeds AR, Emery P, Till SJ, Durham SR. Development and validation of a structured questionnaire for the diagnosis of oral allergy syndrome in subjects with seasonal allergic rhinitis during the UK birch pollen season. Clin Exp Allergy. 2011;41(7):1001-11.
301. Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J Allergy Clin Immunol. 1995;95(5 Pt 1):962-9.
302. Ebo DG, Bridts CH, Verweij MM, De Knop KJ, Hagendorens MM, De Clerck LS, et al. Sensitization profiles in birch pollen-allergic patients with and without oral allergy syndrome to apple: lessons from multiplexed component-resolved allergy diagnosis. Clin Exp Allergy. 2010;40(2):339-47.
303. Osterballe M, Hansen TK, Mortz CG, Bindslev-Jensen C. The clinical relevance of sensitization to pollen-related fruits and vegetables in unselected pollen-sensitized adults. Allergy. 2005;60(2):218-25.
304. Pickert CN, Lorentz A, Manns MP, Bischoff SC. Colonoscopic allergen provocation test with rBet v 1 in patients with pollen-associated food allergy. Allergy. 2012.
305. Tobin MC, Moparty B, Farhadi A, DeMeo MT, Bansal PJ, Keshavarzian A. Atopic irritable bowel syndrome: a novel subgroup of irritable bowel syndrome with allergic manifestations. Ann Allergy Asthma Immunol. 2008;100(1):49-53.
306. Zar S, Kumar D, Benson MJ. Food hypersensitivity and irritable bowel syndrome. Aliment Pharmacol Ther. 2001;15(4):439-49.
307. Goral V, Kucukoner M, Buyukbayram H. Mast cells count and serum cytokine levels in patients with irritable bowel syndrome. Hepato-gastroenterology. 2010;57(101):751-4.
308. O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, et al. Increased mast cells in the irritable bowel syndrome. Neurogastroenterol Motil. 2000;12(5):449-57.

309. Santos J, Guilarte M, Alonso C, Malagelada JR. Pathogenesis of irritable bowel syndrome: the mast cell connection. Scand J Gastroenterol. 2005;40(2):129-40.
310. Homsak M, Silar M, Berce V, Tomazin M, Skerbinjek-Kavalar M, Celesnik N, et al. The Relevance of Basophil Allergen Sensitivity Testing to Distinguish between Severe and Mild Peanut-Allergic Children. Int Arch Allergy Immunol. 2013;162(4):310-7.
311. Glaumann S, Nopp A, Johansson SG, Borres MP, Nilsson C. Oral peanut challenge identifies an allergy but the peanut allergen threshold sensitivity is not reproducible. PLoS One. 2013;8(1):e53465.
312. Keet CA, Johnson K, Savage JH, Hamilton RG, Wood RA. Evaluation of Ara h2 IgE thresholds in the diagnosis of peanut allergy in a clinical population. The journal of allergy and clinical immunology in practice. 2013;1(1):101-3.
313. Eigenmann PA, Burks AW, Bannon GA, Sampson HA. Identification of unique peanut and soy allergens in sera adsorbed with cross-reacting antibodies. J Allergy Clin Immunol. 1996;98(5 Pt 1):969-78.
314. Ballmer-Weber BK, Vieths S. Soy allergy in perspective. Curr Opin Allergy Clin Immunol. 2008;8(3):270-5.
315. Ballmer-Weber BK, Holzhauser T, Scibilia J, Mittag D, Zisa G, Ortolani C, et al. Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol. 2007;119(6):1489-96.
316. Klemans RJ, Knol EF, Michelsen-Huisman A, Pasmans SG, de Kruijf-Broekman W, Bruijnzeel-Koomen CA, et al. Components in soy allergy diagnostics: Gly m 2S albumin has the best diagnostic value in adults. Allergy. 2013;68(11):1396-402.
317. Mittag D, Vieths S, Vogel L, Becker WM, Rihs HP, Helbling A, et al. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. J Allergy Clin Immunol. 2004;113(1):148-54.
318. Holzhauser T, Wackermann O, Ballmer-Weber BK, Bindslev-Jensen C, Scibilia J, Perono-Garoffo L, et al. Soybean (Glycine max) allergy in Europe: Gly m 5 (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. J Allergy Clin Immunol. 2009;123(2):452-8.
319. Javaloyes G, Goikoetxea MJ, Garcia Nunez I, Sanz ML, Blanca M, Scheurer S, et al. Performance of different in vitro techniques in the molecular diagnosis of peanut allergy. J Investig Allergol Clin Immunol. 2012;22(7):508-13.
320. Woods RK, Stoney RM, Raven J, Walters EH, Abramson M, Thien FC. Reported adverse food reactions overestimate true food allergy in the community. Eur J Clin Nutr. 2002;56(1):31-6.

321. Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. Ann N Y Acad Sci. 2002;964:47-68.
322. Eriksson NE, Moller C, Werner S, Magnusson J, Bengtsson U, Zolubas M. Self-reported food hypersensitivity in Sweden, Denmark, Estonia, Lithuania, and Russia. J Investig Allergol Clin Immunol. 2004;14(1):70-9.
323. Lieberoth S, Backer V, Kyvik KO, Skadhauge LR, Tolstrup JS, Gronbaek M, et al. Intake of alcohol and risk of adult-onset asthma. Respir Med. 2012;106(2):184-8.
324. Linneberg A, Berg ND, Gonzalez-Quintela A, Vidal C, Elberling J. Prevalence of self-reported hypersensitivity symptoms following intake of alcoholic drinks. Clin Exp Allergy. 2008;38(1):145-51.
325. Vally H, Thompson PJ. Allergic and asthmatic reactions to alcoholic drinks. Addiction biology. 2003;8(1):3-11.
326. Ronmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundback B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. J Allergy Clin Immunol. 2009;124(2):357-63, 63.e1-15.
327. Ekerljung L, Bossios A, Lotvall J, Olin AC, Ronmark E, Wennergren G, et al. Multi-symptom asthma as an indication of disease severity in epidemiology. Eur Respir J. 2011;38(4):825-32.
328. Pallasaho P, Juusela M, Lindqvist A, Sovijarvi A, Lundback B, Ronmark E. Allergic rhinoconjunctivitis doubles the risk for incident asthma--results from a population study in Helsinki, Finland. Respir Med. 2011;105(10):1449-56.
329. Warm K, Backman H, Lindberg A, Lundback B, Ronmark E. Low incidence and high remission of allergic sensitization among adults. J Allergy Clin Immunol. 2012;129(1):136-42.

APPENDIX

Table 1S. Correlations between the most influential variables associated with severe peanut allergy (PA), as revealed by the OPLS-DA analysis, shown in Figure 9B and Figure 10 in the *study II*.

Correlations in the PA-group with the Spearman's Correlation Test

			SPT Peanut	IgE Peanut	IgE Soy	BAT AC50 Peanut	BAT AC50 Soy	BAT AC50 Birch	rAra h 1	rAra h 2	rAra h 3	nAra h 6	nGly m 5	nGly m 6
Spearman's rho	**SPT Peanut**	Correlation Coefficient	1,000	,061	-,057	-,022	-,041	-,100	-,027	,070	,000	,121	-,140	-,114
		Sig. (2-tailed)	.	,682	,705	,882	,787	,505	,855	,639	,999	,420	,349	,447
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	IgE Peanut	Correlation Coefficient	,061	1,000	,770**	,235	,584**	-,044	,846**	,752**	,809**	,784**	,558**	,652**
		Sig. (2-tailed)	,682	.	,000	,112	,000	,769	,000	,000	,000	,000	,000	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	IgE Soy	Correlation Coefficient	-,057	,770**	1,000	,099	,585**	,184	,677**	,612**	,823**	,609**	,659**	,923**
		Sig. (2-tailed)	,705	,000	.	,507	,000	,215	,000	,000	,000	,000	,000	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	BAT AC50 Peanut	Correlation Coefficient	-,022	,235	,099	1,000	,413**	,161	,314*	,291*	,289*	,347*	,036	,068
		Sig. (2-tailed)	,882	,112	,507	.	,004	,280	,032	,047	,049	,017	,813	,652
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	BAT AC50 Soy	Correlation Coefficient	-,041	,584**	,585**	,413**	1,000	,268	,508**	,476**	,661**	,484**	,391**	,583**
		Sig. (2-tailed)	,787	,000	,000	,004	.	,068	,000	,001	,000	,001	,007	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	BAT AC50 Birch	Correlation Coefficient	-,100	-,044	,184	,161	,268	1,000	,086	,107	,130	,108	,221	,304*
		Sig. (2-tailed)	,505	,769	,215	,280	,068	.	,565	,476	,384	,469	,135	,037
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	rAra h 1	Correlation Coefficient	-,027	,846**	,677**	,314*	,508**	,086	1,000	,777**	,796**	,804**	,459**	,603**
		Sig. (2-tailed)	,855	,000	,000	,032	,000	,565	.	,000	,000	,000	,001	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	rAra h 2	Correlation Coefficient	,070	,752**	,612**	,291*	,476**	,107	,777**	1,000	,742**	,929**	,367*	,543**
		Sig. (2-tailed)	,639	,000	,000	,047	,001	,476	,000	.	,000	,000	,011	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	rAra h 3	Correlation Coefficient	,000	,809**	,823**	,289*	,661**	,130	,796**	,742**	1,000	,741**	,632**	,791**
		Sig. (2-tailed)	,999	,000	,000	,049	,000	,384	,000	,000	.	,000	,000	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	nAra h 6	Correlation Coefficient	,121	,784**	,609**	,347*	,484**	,108	,804**	,929**	,741**	1,000	,389**	,547**
		Sig. (2-tailed)	,420	,000	,000	,017	,001	,469	,000	,000	,000	.	,007	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	nGly m 5	Correlation Coefficient	-,140	,558**	,659**	,036	,391**	,221	,459**	,367*	,632**	,389**	1,000	,701**
		Sig. (2-tailed)	,349	,000	,000	,813	,007	,135	,001	,011	,000	,007	.	,000
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47
	nGly m 6	Correlation Coefficient	-,114	,652**	,923**	,068	,583**	,304*	,603**	,543**	,791**	,547**	,701**	1,000
		Sig. (2-tailed)	,447	,000	,000	,652	,000	,037	,000	,000	,000	,000	,000	.
		Number of patients	47	47	47	47	47	47	47	47	47	47	47	47

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 2S. Correlations between the most influential variables associated with peanut sensitization (PS), as revealed by the OPLS-DA analysis, shown in Figure 9B in the *study II.*

Correlations in the PS-group with the Spearman's Correlation Test

			IgE Birch	BAT AC50 Peanut	BAT AC50 Soy	BAT AC50 Birch	rAra h 8	rGly m 4
Spearman's rho	**IgE Birch**	Correlation Coefficient	1,000	,418	,192	,258	,795**	,738**
		Sig. (2-tailed)	.	,053	,391	,246	,000	,000
		Number of patients	22	22	22	22	22	22
	BAT AC50 Peanut	Correlation Coefficient	,418	1,000	,689**	,735**	,479*	,638**
		Sig. (2-tailed)	,053	.	,000	,000	,024	,001
		Number of patients	22	22	22	22	22	22
	BAT AC50 Soy	Correlation Coefficient	,192	,689**	1,000	,442*	,373	,447*
		Sig. (2-tailed)	,391	,000	.	,040	,088	,037
		Number of patients	22	22	22	22	22	22
	BAT AC50 Birch	Correlation Coefficient	,258	,735**	,442*	1,000	,362	,372
		Sig. (2-tailed)	,246	,000	,040	.	,098	,088
		Number of patients	22	22	22	22	22	22
	rAra h 8	Correlation Coefficient	,795**	,479*	,373	,362	1,000	,757**
		Sig. (2-tailed)	,000	,024	,088	,098	.	,000
		Number of patients	22	22	22	22	22	22
	rGly m 4	Correlation Coefficient	,738**	,638**	,447*	,372	,757**	1,000
		Sig. (2-tailed)	,000	,001	,037	,088	,000	.
		Number of patients	22	22	22	22	22	22

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).