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ALLERGY DIAGNOSIS IN CHILDREN IgE-SENSITIZED TO PEANUT

Clinical and Immunological Evaluation

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ALLERGY DIAGNOSIS IN CHILDREN IgE-SENSITIZED TO
PEANUT-Clinical and Immunological Evaluation
THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

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ABSTRACT

Background

Peanut allergy is often life-long and affects quality of life since accidental ingestion may lead to severe or even fatal reactions. Sensitization to peanut can be due to genuine peanut allergy or to cross-sensitization due to birch pollen. Peanut allergy diagnosis is usually based on clinical history, skin prick test (SPT) and presence of IgE-antibodies (IgE-ab) to peanut but these tests often need to be confirmed with an oral food challenge which may cause severe allergic reactions. Measurements of IgE-ab to specific proteins in an allergen source (component resolved diagnostics [CRD]) and basophil allergen threshold sensitivity (CD-sens) may be valuable tools for diagnosis of peanut allergy. Important allergen proteins in peanut are the storage proteins: Ara h 1, Ara h 2 and Ara h 3 and the PR-10 protein [birch-homologue] Ara h 8.

Aim

The aim of this thesis was to evaluate different diagnostic methods in children IgE-sensitized to peanut with a suspected peanut allergy.

Method

Paper I investigated if it is possible to predict the outcome of double-blind placebo-controlled food challenge (DBPCFC) with peanut by measuring CD-sens to peanut and Ara h 2 as well as IgE-ab to peanut components (Ara h 1, Ara h 2, Ara h 3, Ara h 8 or Ara h 9) (n=38). In Paper II, the reproducibility of DBPCFC and CD-sens were investigated. Twenty-seven children underwent DBPCFC followed by a single-blinded food challenge with peanut, and CD-sens was measured before the two first peanut challenges. Paper III reports a birch pollen allergic child with cross-sensitization to peanut who had a severe reaction after eating a large amount of peanuts. The fourth paper investigated the outcome of a peanut challenge in relation to IgG4-ab (n=58). Paper V studied 20 birch pollen allergic children cross-sensitized to peanut in relation to CD-sens to peanut and Ara h 8.

Results

In Paper I, 25 children had a positive DBPCFC and 92% of the children were positive in CD-sens. The remaining two children were low responders and could not be evaluated. Children with positive DBPCFC reactions had significantly higher levels of IgE-ab to peanut, Ara h 1, Ara h 2 and Ara h 3 than children with negative reactions. All children negative in CD-sens to peanut and Ara h 2 were also negative in challenge. In paper II, 14/27 children were positive at both active challenges but not placebo. Only three of these children reacted consistently at the same dose with the same severity score. All children with a positive or a negative CD-sens at the first challenge were also CD-sens positive/negative at the second challenge. Paper III revealed that the girl with birch pollen allergy who reacted with anaphylaxis after peanut ingestion was mono-sensitized to Ara h 8. Paper IV showed that children positive at peanut challenge had significantly higher levels of IgG4-ab to peanut and Ara h 2 than children negative at the challenge. The peanut and Ara h 2 IgG4/IgE-ab ratios were significantly higher in children who tolerated peanut than in allergic children. In Paper V, all children passed peanut challenge without any objective symptoms, but five experienced subjective symptoms from the oral cavity. CD-sens to peanut was negative in 19/20 children but 17/20 were positive in CD-sens to Ara h 8.

Conclusion

CD-sens is a promising diagnostic method with good reproducibility in the diagnosis of peanut allergy and may exclude a peanut allergy. IgE-ab to the peanut storage proteins (Ara h 1, Ara h 2 and Ara h 3) seem to confirm a genuine peanut allergy. A peanut challenge can discriminate between positive and negative reactions but does not predict the severity of an allergic reaction. Birch-pollen allergic children IgE-sensitized to peanut and Ara h 8 but not to Ara h 1, Ara h 2 and Ara h 3 have basophils sensitized with IgE-ab to Ara h 8 which can be activated by Ara h 8 proteins and initiate allergic inflammation. Children IgE-sensitization to peanut who nonetheless tolerate peanuts are characterized by low levels of IgG4-antibodies to peanut and Ara h 2 but relatively high IgG4/IgE antibody ratios.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers which are referred to by their Roman numerals

- I. Glaumann S, Nopp A, Johansson S.G.O., Rudengren M, Borres MP, Nilsson C.**
Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children.
Allergy. 2012 Feb;67(2):242-7
- II. Glaumann S, Nopp A, Johansson S.G.O., Borres MP, Nilsson C.**
Oral peanut challenge identifies an allergy but the peanut allergen threshold sensitivity is not reproducible.
PLoS One. 2013;8(1)
- III. Glaumann S, Nopp A, Johansson S.G.O., Borres MP, Lilja G, Nilsson C.**
Anaphylaxis to peanuts in a 16-year-old girl with birch pollen allergy and with monosensitization to Ara h 8.
J Allergy Clin Immunol Pract. 2013 Nov-Dec;1(6):698-9
- IV. Glaumann S, Nilsson C, Asarnoj A, Movérare R, Johansson S.G.O., Borres M.P, Lilja G, Nopp A.**
IgG₄-antibodies and peanut challenge outcome in children IgE-sensitized to peanut.
In manuscript
- V. Glaumann S, Nilsson C, Johansson S.G.O., Asarnoj A, Wickman M, Borres MP, Nopp A.**
Basophil allergen threshold sensitivity (CD-sens) to peanut and Ara h 8 in children IgE-sensitized to birch and Ara h 8.
Submitted

Other publications related to this project:

Lieberman J, Glaumann S, Båtelson S, Borres MP, Sampson H, Nilsson C.
The utility of peanut components in the diagnosis of IgE-mediated peanut allergy among distinct populations.

J Allergy Clin Immunol: In Practice 2013;1:75-82

Asarnej A, Nilsson C, Lidholm J, Glaumann S, Östblom E, Hedlin G, van Hage M, Lilja G, Wickman M.

Peanut component Ara h 8 sensitization and tolerance to peanut.

J Allergy Clin Immunol. 2012;130(2):468-72

Asarnej A, Glaumann S, Elfström L, Lilja G, Lidholm J, Nilsson C, Wickman M.

Anaphylaxis to peanut in a patient predominantly sensitized to Ara h 6.

Int Arch Allergy Immunol. 2012;159(2):209-12

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LIST OF ABBREVIATIONS

APC	Antigen presenting cell
BAT	Basophil activation test
BcR	B-cell receptor
CD	Cluster of differentiation
CD-sens	Basophil allergen threshold sensitivity
CRD	Component resolved diagnostics
DBPCFC	Double-blind placebo-controlled food challenge
Fab	Fragment antigen binding
Fc	Fragment crystallizable
FPIES	Food protein induced enterocolitis syndrome
HSC	Hematopoietic stem cell
IgE-ab	IgE-antibody
IgG ₄ -ab	IgG ₄ -antibody
IL	Interleukin
LC	Lowest concentration
LLOQ	Lower limit of quantification
LTP	Lipid transfer proteins
MA	Molecular-based allergy
NK-cell	Natural killer cell
OAS	Oral allergy syndrome
OFC	Open food challenge
PBS	Phosphate buffered saline
PFS	Pollen-food allergy syndrome
PR	Pathogenesis-related
SBFC	Single-blind food challenge
SPT	Skin prick test
T _H -cell	T-helper cell
TNF- α	Tumor necrosis factor- α

1 INTRODUCTION

Peanut allergy is a potentially life-threatening condition of unprecedented complexity and severity (1). Symptoms vary among individuals and accidental ingestion of peanuts may result in anaphylactic reaction, which can be fatal (2-7). A peanut allergic individual needs to avoid a wide variety of foods containing peanuts, including products with precautionary labeling (8). Due to risk of severe reactions adrenaline auto-injectors are often prescribed. All aspects associated with fear of an anaphylactic reaction can impair quality of life (7, 9-12).

Individuals IgE-sensitized to peanuts are often recommended to avoid peanuts irrespective of whether they have experienced allergic symptoms or not (11, 13). Therefore an accurate diagnosis is highly important to avoid serious consequences for the affected child and his or her family. A peanut allergy diagnosis is usually based on clinical history, skin prick test (SPT) and presence of IgE-antibodies (IgE-ab) to peanut in serum. However, the diagnosis often needs to be confirmed with an oral food challenge, preferably a double-blind placebo-controlled food challenge (DBPCFC), which is the gold standard (14). Oral food challenges are time consuming and pose a risk that the individual will develop a severe allergic reaction. Therefore oral challenges often need to be performed by specialized personnel who can handle that type of emergency. There are now other promising diagnostic methods, such as component resolved diagnostics (CRD) and CD-sens (basophil allergen threshold sensitivity). CRD involves investigating the presence of antibodies to different peanut allergen proteins. CD-sens evaluates allergen threshold sensitivity of basophils by using a dose-response curve measuring percentage of activated basophils at different concentration. This method has been shown to correlate with asthma sensitivity and allergic rhinitis (15-18).

The aim of this thesis was to evaluate different diagnostic methods in children IgE-sensitized to peanut with a suspected diagnosis of peanut allergy. The terminology used in this thesis adheres to the recommended nomenclature for allergy from WAO 2003 (19, 20).

2 BACKGROUND

2.1 FOOD ALLERGY

Food allergy is a common disease in childhood. The condition is estimated to affect 12% of children in Western countries when assessed from self-reported symptoms and approximately 3% when based on clinical history or DBPCFC (21). However, prevalence reports vary worldwide depending on differences in allergy definitions, study populations, geographic variations and dietary exposure (14, 21-23). Patients often confuse food intolerance with food allergies and there is an unfounded belief that food allergy is more prevalent than it actually is. An adverse food reaction can either be immune mediated (food allergy) or non-immune mediated (food intolerance) as shown in Figure 1 (24).

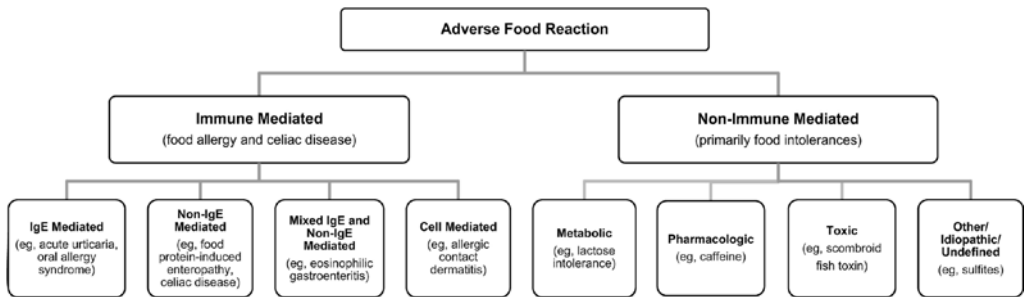


Figure 1. Types of adverse reactions to food. With permission from Boyce et al (24).

Food intolerance is more common than genuine food allergy and is caused by the pharmacological properties of the food, or by defects in metabolism of certain food components (25). Examples of food intolerance are lactose intolerance and toxic reactions, such as scombroid food poisoning (from eating spoiled fish). Sometimes food intolerance reactions mimic an immune mediated reaction with symptoms such as vomiting, flushing, and stomach ache shortly after ingestion. An immune mediated food allergy is defined as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food”. This definition includes cell mediated, IgE-mediated, non IgE-mediated or a combination of both IgE-mediated and non IgE-mediated allergy.

Example of a non IgE-mediated reaction is food protein induced enterocolitis syndrome (FPIES) which can present with acute symptoms such as repetitive emesis and dehydration a few hours after exposure. Eosinophilic esophagitis is a combination of both IgE-mediated and non IgE-mediated reactions and involves localized eosinophilic inflammation in the esophagus. Allergic contact dermatitis is caused by cell mediated reactions to chemical haptens that are additives to foods or occur naturally in foods, such as mango (24).

This thesis focuses on IgE-mediated food allergy, which is the most common cause of food allergy in children. Over 170 foods have been reported to cause an IgE-mediated allergy; however, by far most common foods to cause allergic reactions are milk, egg, soy, wheat, peanut, tree nut, fish and shellfish (8, 24). A food allergy is seldom an isolated phenomenon and children with allergy to milk and/or egg in early infancy often develop additional food allergies, e.g. peanut (26). However, in general, it is more likely that allergies to milk, egg, soy and wheat resolve during childhood whereas allergy to peanut, tree nuts, fish and shellfish is persistent (14).

In IgE-mediated allergy the immune system recognizes an otherwise harmless substance as foreign (allergen). The immune system starts to produce IgE-ab and the individual get sensitized to the specific allergen. Being sensitized to an allergen is not equal to being allergic (27, 28). However, the probability of an allergic reaction increases with increasing concentrations of IgE-ab but the levels of IgE-ab do not seem to predict the severity of an allergic reaction (8, 29).

There are several proposed risk factors influencing food allergy and IgE-sensitization (30). Boys have a higher risk of developing food allergy (31), suggesting genetic or endocrinologic influences and a child with an allergic parent or sibling has an increased risk to become allergic to food (30). Atopy, defined as *a personal/familial tendency to produce IgE-ab to otherwise harmless substances*, is also a known risk factor for developing food allergy (19). Other suggested risk factors are vitamin D insufficiency, low consumption of

antioxidants, certain timings and routes of exposure to foods and obesity (14, 30, 31).

2.2 IMMUNOLOGY

2.2.1 Overview of the immune system

The human immune system protects the body against pathogens that range in size from small intracellular viruses to large parasitic organisms and has developed a wide range of recognition and destruction mechanisms to combat these diverse potential invaders (32, 33). An allergic reaction occurs if the immune system recognizes an otherwise harmless substance as foreign (allergen) and starts an immune response against the allergen (27).

Different kinds of white blood cells are needed to produce an intact immune response and all these cells arise from a single cell type, hematopoietic stem cell (HSC) found in the bone marrow. Figure 2 shows important immune cells derived from the HSC.

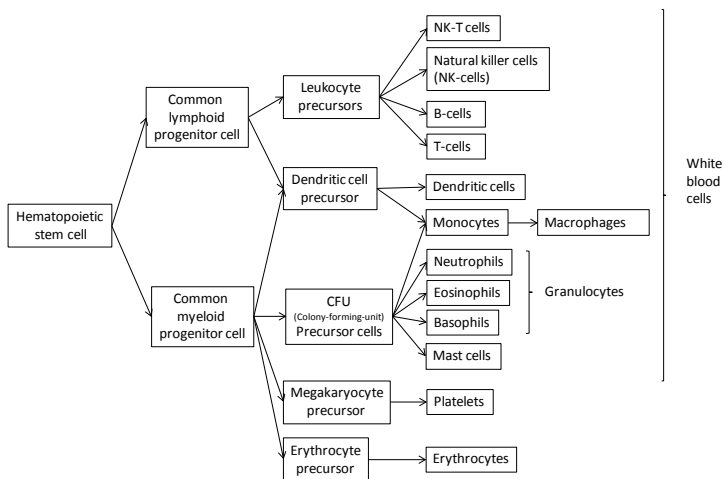


Figure 2. Hematopoiesis.

Self-renewing hematopoietic stem cells give rise to all cells involved in an immune response. Most cells develop in the bone marrow and then travel to peripheral lymphoid organs. Mast cells and macrophages continue to mature outside the bone marrow and T-cells mature in the thymus. Modified from Chaplin et al and Owen et al (32, 34).

Granulocytes play an important role in the immune response. Neutrophils have a large phagocytic capability and accumulate in large quantities at sites of infection. They produce reactive oxygen that is cytotoxic to bacterial pathogens and the cells are also part of tissue remodeling following injury. Eosinophils, basophils and mast cells contain mediators that are released upon activation and are known to protect the host from parasites and helminths but they are also prominent cells in most allergic responses. Monocytes and macrophages are highly phagocytic and are mobilized to the site of infection shortly after the neutrophils. They often persist at the site of infection for a long time and produce nitric oxide as a major mechanism of killing. Activated macrophages produce large amounts of proinflammatory cytokines (34).

B-cells are the antibody-producing cells in the immune system. All B-cells express a membrane bound immunoglobulin molecule (with or without antibody activity): the B-cell receptor (BcR). The BcR has a unique antigen binding specificity and activated B-cells can produce large amounts of antibodies with an antigen-binding site identical to the BcR (32, 34). The immunoglobulins share a common structure of two identical light chains and two identical heavy chains and form a Y shape. (Figure 3) (35). All immunoglobulins are functionally divided into two fragments, the Fab fragment and the Fc fragment. The Fab fragment is the antigen binding site which is unique for each antibody. The base

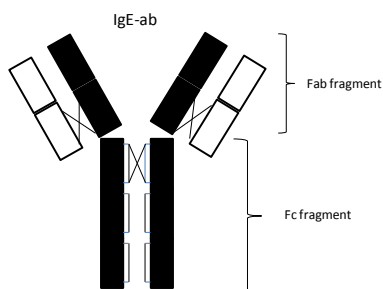


Figure 3. Structure of IgE-ab.
 Heavy chain (black), light chain (white)
 Modified from Gould (35).

of the antibody is called the Fc region. It is identical for all antibodies of a given class and binds into Fc receptors found on different kinds of effector cells. There are five classes of immunoglobulins (IgA, IgD, IgG, IgE and IgM) with different functions in the immune system. In the circulation the immunoglobulin of a certain class can be bound together in complex (IgM circulates as a pentamer and IgA as a dimer).

Depending on the cytokine milieu surrounding them, activated B-cells change their production of immunoglobulins from one class to another (class-switch) (34, 36).

T-cell derived cytokines induce class-switch of the immunoglobulins produced by B-cells during an immune response. In the thymus T-cells mature into two major subpopulations, cytotoxic T-cells and T-helper cells (T_H -cells). Cytotoxic T-cells are part of the cell mediated immune response to kill infected cells, while T_H -cells are involved in activation of B-cells (32, 33). The largest group of T-cells is the T_H -cells. T_H -cells can be activated by macrophages, dendritic cells and activated B-cells (antigen presenting cells [APC]). Depending on type of cytokine milieu at site of infection the T_H -cells differentiate into different subpopulations and secrete a unique mixture of cytokines with distinct effector functions (33, 37). The different kinds of T_H -cells are shown in Figure 4.

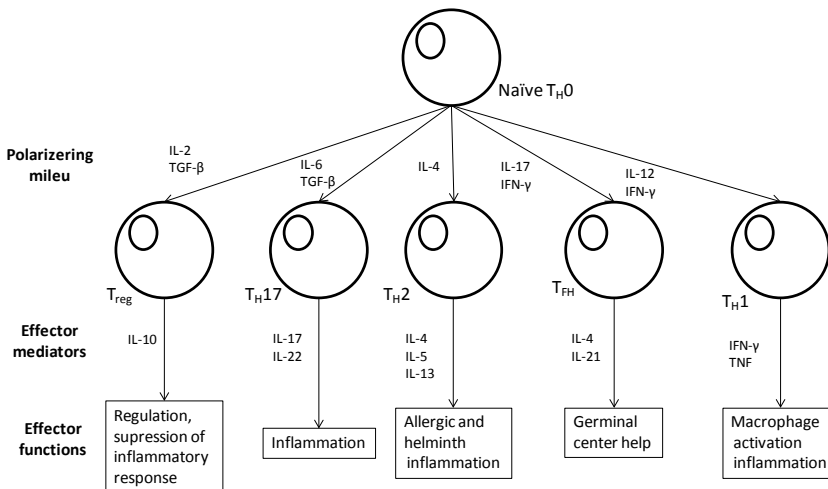


Figure 4. Subsetting T-cell responses based on T_H -cell polarization.

T-cells becomes polarized into different effector T_H -cells depending on polarizing cytokine milieu when activated. Modified from Swain et al (37).

2.2.2 Immune cells and antibodies

Mast cells

Mast cells are primarily located in the tissue near blood vessels and epithelial surfaces and are long-lived cells that can stay in the tissue for months. They are one of the major effector cells in IgE-mediated allergy but also of importance in long term pathophysiological changes and tissue remodeling associated with chronic inflammation (38). The mast cells express the activating receptor for IgE-ab (FcεRI) which is up-regulated in presence of increased concentrations of free IgE-molecules circulating in the blood. The biological function of mast cells is unknown (39). It has been suggested that these cells are involved in regulation of wound healing and protection from severe bacterial and parasitic skin infections and from reactions after severe venom insects and snake bites (40).

Basophils

Like mast cells, basophils play a role in allergic inflammation and the two cell types share several features, such as expression of FcεRI, secretion of T_H2-cytokines and histamine release on activation. Basophils are found in the circulation and represent <1% of peripheral blood leukocytes (41, 42). The life-span of the basophils is short, approximately 60 hours, but increases during inflammation (43). The percentage of circulating basophils among different individuals varies but there is currently no evidence that an increased number of circulating basophils correlates with allergic diseases (44). Even though the basophils share many features with mast cells, they represent a distinct cell lineage and are not a population of immature circulating mast cells (45). Basophils are known to have a strong association with helminth infections but also with T_H2 associated diseases. They are involved in delayed-type hypersensitivity reactions in humans; they infiltrate the tissue following acute allergic reactions and are for example found in the lungs in severe asthma, in the upper respiratory tract in individuals with allergic rhinitis and in the skin of individuals with atopic eczema (44). The basophils also represent an important

source of interleukin (IL)-4 / IL-13 and secrete histamine, proteases and leukotrienes -all of central importance in promoting allergic inflammation and allergic disease (42, 44, 46, 47).

IgE-antibodies

IgE was discovered simultaneously in 1967 by one Swedish and one American research group working independently (48, 49). The discovery was a breakthrough and had great impact on our understanding of the immunological basis of allergic diseases (50).

IgE-ab is an important mediator in allergic inflammation and its production is promoted by IL-4/IL-13, both of which are produced during a T_H2-dependent response. The concentration of IgE in serum is low compared to other immunoglobulins and its half-life is short, approximately two days. Expression of IgE-ab is normally strictly regulated but the concentration is elevated in atopic and allergic individuals (46). There are two IgE-receptors: the low affinity receptor FcεRII (CD23) and the high affinity receptor FcεRI. The latter is expressed on both mast cells and basophils and has a crucial role in allergic inflammation. The half-life of an unbound FcεRI on a mast cell is 24 hour *in vitro*. However, the *in vitro* half-life of the IgE-FcεRI complex is considerably longer and it appears to be expressed on the mast cells throughout the whole life span of the cell. The low affinity receptor (FcεRII) is expressed on B-cells and other hematopoietic cells, for example APC and intestinal epithelial cells. Activation of the low affinity receptor leads to regulation of IgE-production, killing of intracellular pathogens and facilitation of antigen presentation (38, 46).

IgG₄-antibodies

IgG is the most common immunoglobulin in human. It is divided into four subclasses (IgG₁, IgG₂, IgG₃, IgG₄) of which IgG₄ is the least abundant (<5%). The association of IgG₄ with IgE-mediated allergic inflammation is known, but its exact role is poorly understood (51). It has been hypothesized that IgG₄-ab act

as blockers, binding to the allergen and inhibiting binding between the allergen and IgE-ab (52, 53). IgG₄-ab can also bind to the low affinity IgG-receptor (FcγIIB) on mast cells and basophils, leading to inhibition of cell degranulation (54, 55).

2.2.3 IgE-mediated allergic inflammation

In IgE-mediated allergy, the immune system responds to an otherwise harmless substance in an inappropriate way involving both T_H2-cell response and IgE-ab. The reaction resembles the immune response to helminths and parasites which has led to the idea that the immune system is deceived to react to otherwise harmless antigens in the same way as it responds to helminth infections. After the individual has been sensitized and started to produce IgE-ab, an allergic reaction can occur. The reaction is divided into an acute IgE-mediated response and a late phase response. After the initial exposure (sensitization) the immune system has primed mast cells and basophils with IgE-ab and they are ready to start an allergic inflammation if re-exposed to the allergen (22, 27, 56).

Sensitization and the acute phase of an allergic reaction are described in Figure 5. Some individuals also develop a late phase response which typically develops two to six hours after the initial reaction (57). During the IgE-ab mediated activation, mast cells and basophils produce a wide range of cytokines, chemokines and growth factors but these are released more slowly than the preformed mediators. The newly produced cytokines facilitate the influx of more T_H-cells (which change the cytokine environment), monocytes, eosinophils, basophils and neutrophils, causing mucus secretion, vasodilatation, increased vascular permeability, constriction of the bronchi and tissue remodeling (22, 57). The late phase response often peaks after six to nine hours.

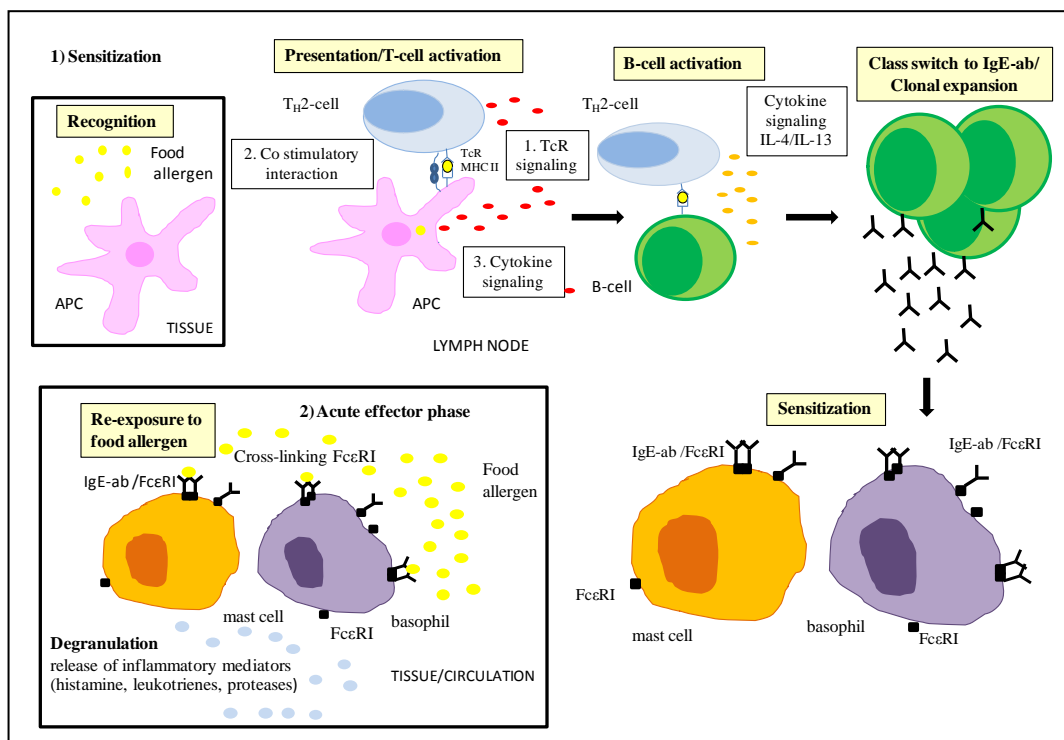


Figure 5. Mechanism of acute IgE-mediated allergic inflammation.

1) Sensitization: The allergen penetrates the mucosa and is internalized by antigen presenting cells (APC) through phagocytosis. The allergen is degraded to peptides in the APC and presented to the T_H2 -cells through MHC II molecules on the cell surface. Activation of the naïve T_H2 -cell occurs if the T_H2 -cell is activated by three signals: 1) Recognition between the TcR and the MHC II molecule. 2) Co-stimulatory interaction between T-cell and APC. 3) Paracrine secretion of cytokines by APC. Once activated, the effector T_H2 -cell activates the B-cell and secretes cytokines (IL-4/ IL-13) which induces class switch in B-cells to IgE-ab production. IgE-ab binds to high affinity receptor (FcεRI) on mast cells and basophils for a faster response on re-exposure to the allergen.

2) Acute effector phase: On re-exposure to the allergen, cross-linking of the FcεRI-IgE-ab complex binds to the allergen and activates mast cells and basophils, which then release mediators (e.g. histamine, cytokines, leukotrienes, prostaglandins, proteolytic enzymes) leading to inflammation and tissue damage (22, 27, 56).

2.3 PLANT FOOD ALLERGENS

Allergen proteins are classified into families and super-families according to structural and functional features. Many food allergens belong to the cupin and prolamin super-families. The cupin super-family (7S and 11S storage seeds proteins) is divided into vicilin and legumin protein families where soybean, peanut and tree nut seed storage proteins are found. The prolamin super-family is divided into three major groups: the seed storage 2S albumins found in tree nuts and seeds, the non-specific lipid transfer proteins (LTP) found in soft fruits and vegetables and cereal α -amylase/trypsin inhibitors (58, 59).

The pathogenesis-related proteins (PRs) are a heterogenous collection of 14 plant protein families which are involved in plant resistance to pathogens. Many plant food allergens are homologous to PRs (59). The major birch pollen allergen, Bet v 1, is a member of the PR-10 family and birch pollen allergic individuals may experience symptoms from the oral cavity known as oral allergy syndrome (OAS) when they eat certain plant foods. The majority of these reactions occur after consumption of allergens of the Rosaceae fruits (e.g. apple, apricots and pears) or Apiaceae vegetables (e.g. celery and carrots), which cross-react with allergens presented in pollen from birch (Bet v 1) and other trees. Peanut contains Ara h 8, which is a Bet v 1 related allergen and a member of the PR-10 family (58). This is the reason why birch pollen allergic individuals also may experience OAS when eating peanuts.

2.4 PEANUT ALLERGY

Peanut allergy is a serious health concern affecting both children and adults. Despite the risk for severe reaction there is currently no treatment; instead a strict diet without peanuts is recommended (60, 61). Peanut allergy is also often persistent. Only 20% of the individuals allergic to peanut grow out of their allergy and even if they do, it sometimes recurs (61). Co-morbidity with other allergy diseases is common and less than 5% of patients have mono-sensitization to peanut. Peanut allergic individuals are also often atopic and their rates of

asthma, atopic eczema and rhinitis are higher than in the general population (1, 61, 62).

Peanut sensitization is common and several studies indicate that the prevalence is rising (14, 63-67). IgE-sensitization to peanut varies in different studies, from 1% -11% in Western countries and for challenge-proved peanut allergy the prevalence ranges between 0.2 and 1.6% (21, 68, 69)

Clinical presentation and severity of peanut allergy

Symptoms of an IgE-mediated peanut allergy can occur within minutes after exposure, but the reaction can also have a slower onset with up to two hours

Table 1. Symptoms in IgE-mediated allergy reaction.
With permission from Boyce et al (24).

Target organ	Immediate symptoms	Delayed symptoms
Cutaneous	Erythema Pruitus Urticaria Morbilliform eruption Angioedema	Erythema Flushing Pruitus Morbilliform eruption Angioedema Eczematous rash
Ocular	Pruitus Conjunctival erythema Tearing Periorbital edema	Pruitus Conjunctival erythema Tearing Periorbital edema
Upper respiratory	Nasal congestion Pruitus Rhinorrhea Sneezing Laryngeal edema Hoarseness Dry staccato cough	
Lower respiratory	Cough Dyspnea Wheezing Intercostal retraction Accessory muscle use	Cough Dyspnea Wheezing
Gastrointestinal (oral)	Angioedema of the lips, tongue or palate Oral pruritus Tongue swelling	
Gastrointestinal (lower)	Nausea Colicky abdominal pain Reflux Vomiting Diarrhea	Abdominal pain Reflux Vomiting Diarrhea Hematochezia Irritability and food refusal with weight loss (young children)
Cardiovascular	Tachycardia (occasionally) Bradycardia in anaphylaxis Hypotension Dizziness Fainting Low of consciousness	
Miscellaneous	Uterine contraction Sens of "impending doom"	

delay (24). Symptoms range from mild to life-threatening anaphylactic reaction. Common symptoms are listed in Table 1. Acute urticaria and angioedema are common clinical manifestations which may be triggered by ingestion or by direct contact with the skin, the latter causing acute contact urticaria. Even though hives and swelling are common symptoms of an allergic reaction, severe reactions do not necessarily include urticaria. Twenty percent of anaphylactic reactions

do not involve the skin (70). Symptoms from the lower airways, presenting as cough, wheezing and/or dyspnea, could be a sign of a more general allergic reaction. Rhinoconjunctivitis is often observed but rarely as the only presenting symptom (71). Gastrointestinal manifestations are often associated with IgE-mediated food allergy and include pruritus and swelling of the mouth, abdominal pain or cramps, vomiting and diarrhea (24). Upper gastric manifestations often occur early after ingestion whereas low gastric symptoms may be delayed up to two to six hours (71).

Anaphylaxis is a severe and potentially life-threatening systemic allergic reaction. It is defined as presence of symptoms from two or more organ systems after exposure to a likely allergen or hypotension alone after exposure to a known allergen. Anaphylaxis can be biphasic in up to 20% of the reactions and this pattern is more common in severe cases (72). Even though fatal reactions are uncommon they pose a potential risk, especially for teenagers and young adults with co-morbidity of asthma and peanut or tree nut allergy (3, 73). Other related factors often associated with near fatal or fatal reactions are delayed treatment with epinephrine, absence of skin symptoms, patient denial of symptoms and concomitant use of alcohol (2, 3, 6, 71, 74). Foods are the most common cause of anaphylactic reactions (75). This is in line with a Swedish population-based case study investigating anaphylaxis among children 0-18 years at emergency departments in Stockholm. The authors concluded that food was the triggering factor in 92% of the cases and that tree nuts, peanuts, egg and milk were the foods that most frequently elicit anaphylaxis in children (76).

Oral allergy syndrome (OAS) is an IgE-mediated reaction, typically observed in patients allergic to pollen, after ingestion of certain fresh fruits, vegetables, peanuts and tree nuts (77, 78). Symptoms such as itching, stinging pain and vascular edema are restricted to the oral cavity. Itching in the ears and tightness of the throat may occur but the symptoms usually gradually resolve after exposure without treatment (78). OAS is sometimes referred to by the more specific term pollen-food allergy syndrome (PFS) (79, 80).

2.5 COMMON DIAGNOSTIC METHODS IN PEANUT ALLERGY

The first step when diagnosing a peanut allergy is to take a detailed medical history and do a physical examination. It is important to exclude other causes of an adverse reaction than IgE-mediated allergy (Figure 1). If the clinical history provides a strong suspicion of IgE-mediated allergy, laboratory methods can be used to identify presence of IgE-abs. The most widely recommended diagnostic methods are skin prick test (SPT), IgE-ab measurements in serum and oral food challenges (8, 24, 81-83). During the last decade, new diagnostic methods have become available, such as CRD and CD-sens.

2.5.1 Skin prick test

SPT is a quick, inexpensive test utilizing the degree of cutaneous reactivity as a marker for sensitivity. When an allergen is inserted into the skin in a sensitized individual, IgE-ab bound to the mast cells are cross-linked and histamine and other mediators are released. This produces a wheal and flare response (84). A SPT is considered positive if the wheal diameter is larger than 3 mm. Compared to oral challenges, the pooled sensitivity for SPT to peanut is estimated to 95% (88-98%) and the specificity to 61% (47-74%) (85). However, in pollen related food allergy (e.g. celery, carrots, cherries and hazelnuts) the sensitivity of SPT is much lower (20-65%) (86). The source of the allergen used for SPT may also influence the wheal size. Different batches of commercial allergen extracts may contain different amounts of protein, affecting the allergenicity and the potency of the extract (84, 86, 87). However, it is also possible to perform a prick-prick test where native allergens (e.g. a fresh fruit) are used instead of commercial extracts. The procedure of a prick-prick test is: one prick in the fresh food and then a prick in the patient's skin (88). The overall concordance between a positive prick-prick test and a food challenge was 92% when fresh food was used in comparison with 59% with commercial extracts (89).

2.5.2 IgE-ab assays

IgE-ab assays detect and measure circulating IgE-ab that binds to specific allergens in a patient's serum. The IgE-ab assay is described in Figure 6. There are different detection systems for IgE-ab in serum, e.g. HYTEC-288 (HycorBiomedical), Immulite (Siemens) and ImmunoCAP (Phadia). The amount of IgE-ab bound in the assay is reported in arbitrary mass units (kilo international units of allergen specific antibodies per unit volume of sample [kU_A/L]).

In the ImmunoCAP system, one international unit is equal to 2.42 ng of IgE-ab. No conversion ratio has been established in the other systems (90, 91). The

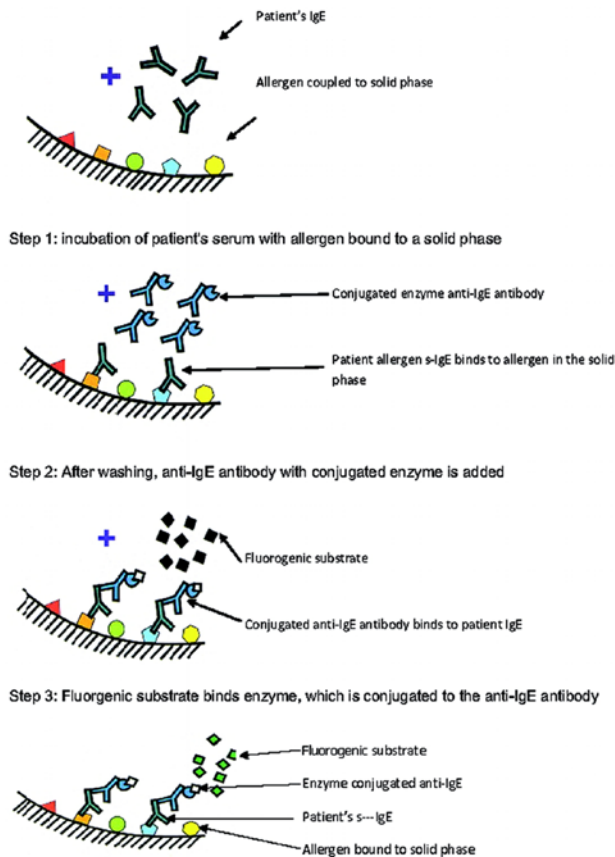


Figure 6. Basic principles for IgE-ab assays.
With permission from Cox et al (91).

analytical sensitivity (lower limit of quantification [LLOQ]) is today 0.1 kU_A/L in all three systems but it should be pointed out that a result from an allergen in one system is not equivalent in another system (92). The pooled sensitivity to peanut IgE-ab is 96% (92-98%) and the pooled specificity is 59% (45-72%) compared to oral challenges. The results obtained with pooled sensitivity and specificity are similar to those from SPT, with a high sensitivity but poor specificity (85).

SPT vs. IgE-ab assay

Serum IgE-ab assays and SPT have similar diagnostic properties. They can both detect sensitization to an allergen. There is also a correlation between increasing concentration of IgE-ab in serum and SPT wheal size and the probability to react to the ingested food (8, 23, 29, 93).

Compared to SPT, IgE-ab assays are a more expensive method but on the other hand the IgE-ab assay does not interfere with severe eczema and the test result is not affected by antihistamine intake (92, 94). Another disadvantage for SPT is that the results may differ depending on use of different skin test devices and techniques (95).

Peanut allergen extract

Peanut extract is commonly used for SPT or IgE-ab assay in the diagnostic work-up for peanut allergy. A benefit of using natural extracts are that ideally all allergenic proteins are present but allergen extract has two main disadvantages. First it is difficult to standardize; natural variability makes extracts differ in allergenicity (28, 96, 97). Second, peanut allergen extract does not differentiate between primary sensitization and immunological cross-reactivity (98).

2.5.3 Oral food challenge

An oral food challenge is performed to make an accurate diagnosis of adverse reaction to foods (99-103). The challenge can be performed open or blinded and the double-blind placebo-controlled food challenge (DBPCFC) is the gold standard. Open food challenge (OFC) is an unmasked challenge with foods in their natural form. An OFC saves time compared to DBPCFC and is often used in clinical practice, particularly since 2/3 of all food challenges are negative (99, 100). A negative OFC can rule out an adverse reaction but a positive test needs to be confirmed with a blinded challenge. A blinded challenge can either be a single-blind food challenge (SBFC) or a DBPCFC. In a SBFC the personnel but not the patients know whether active or placebo food is being served. DBPCFC

is often used for research purposes or when an OFC is not sufficient to rule out a food allergy. At a DBPCFC neither the patient/family nor the personnel know whether the active or placebo food is being served (99, 101).

A food challenge should be performed under medical supervision and without any medication that can mask symptoms, e.g. oral steroids or antihistamines. Challenges with high risk for a reaction should be done in hospital settings with intravenous access. The start dose of a challenge should be low (<100 mg), followed by increasing doses every 20-30 minutes. The challenge should be stopped when objective symptoms occurs (101). A scoring system for severity of food challenge outcome has been recommended in the consensus report for standardizing of oral food challenge from 2012 (101). Unfortunately, this scoring system does not combine the severity of the challenge reaction and the amount of peanut eaten.

2.6 COMPONENT RESOLVED DIAGNOSTICS

CRD, also known as molecular-based allergy (MA) diagnosis, involves using purified, native or recombinant allergen to detect IgE-sensitization to different proteins in an allergen source (104). An allergen source (e.g. crude peanut) contains many different proteins and some of them are associated with allergic reactions. However, many different proteins share common epitopes (binding site for ab) and the same IgE-ab can bind and cause an immune response to proteins with similar structures from other sources (cross-reaction). The stability of a protein also differs during exposure to heat and digestion, which explain why some allergens are tolerated raw while others need to be cooked to be tolerated (98, 104). Proteins in an allergen extract are defined as a major allergen if IgE-ab binds to the protein in more than 50% of the patients with the same allergy. A primary allergen is the original sensitization molecule, in contrast to secondary sensitization caused by cross-reactivity (104). Through knowledge about different protein structure and protein stability it might be possible to differentiate genuine allergic reactions from cross-reactive sensitization (98,

104). Allergenic proteins are designated by their Latin name (genus and species). For example, an allergen that come from *Arachis hypogaea* (peanut) is named Ara h and a number is used to distinguish various allergens from the same species (e.g. Ara h 1, Ara h 2, Ara h 3) (104).

Peanut components

Twelve peanut allergen proteins have so far been discovered and all are listed in the International Union of Immunological Societies Allergen Nomenclature Subcommittee Database (www.allergen.org) (59, 105, 106). Six of these peanut allergens are investigated in this thesis and type of allergen, biological function and known cross-reactivity are listed in Table 2.

Table 2. Peanut allergens

*LTP Lipid transfer protein. Modified from Bublin et al (105).

Protein superfamily	Cupin		Prolamin		Bet v 1 like	
Protein family	Vilicin or 7S Globulin	Legumin or 11S Globulin	2S Albumins		LTP*	Bet v 1 family
Allergen	Ara h 1	Ara h 3	Ara h 2	Ara h 6	Ara h 9	Ara h 8
Biological function	provide nourishment for the growth of seedlings		sources of amino acid for growth of seedlings, involved in defense against pathogens		involved in defense against pathogens and in the formation of hydrophobic layers in plants	might serve as a delivery vehicle for flavonoids
Cross-reactivity	with other legumes and tree nuts vilicins, and Ara h 2 and Ara h 3	with other legumes and tree nuts legumin and Ara h 1, Ara h 2 and Ara h 6	with 2 S albumins from almond, brazil nut, and Ara h 1, Ara h 3 and Ara h 6.	with Ara h 1, Ara h 2 and Ara h 3	with peach and hazelnut LTPs (Pru p 3 and Cor a 8)	with Bet v 1 and other PR-10 proteins e.g. soy and lentil

The clinically most important peanut proteins were discovered by Burks and colleagues. Ara h 1 and Ara h 2 were identified in the beginning of 1990s and Ara h 3 and Ara h 8 were discovered a couple of years later (107-110). The major proteins causing severe allergic reactions are Ara h 1, Ara h 2 and Ara h 3 (111, 112). However, there are geographic differences and all patients do not necessarily react similarly or recognize the same allergens (113). IgE-sensitization to peanut due to cross-sensitization between the major birch pollen allergen Bet v 1 and the peanut allergen Ara h 8 is common in birch-rich areas (68, 69, 113) whereas sensitization to Ara h 9, a member of the LTP allergen

family, plays an important role in patients from the Mediterranean area (113, 114). The known peanut allergen classes constitute 85% of the total protein content of peanut and most (75%) consists of Ara h 1, Ara h 2 and Ara h 3 proteins (115).

2.7 BASOPHIL ACTIVATION

Basophil activation test (BAT) is a functional test investigating basophil activation after exposure to an allergen (116, 117). Basophil function can be tested by using two different methodologies. It is possible to investigate secretion of mediators from basophils (mediator release assays) or by detecting expression of cellular markers after stimulation (flow cytometric assays). The most well-known method of testing mediator release is the *histamine release test* which relies on presence of preformed histamine in granules of basophils. The basophil response is quantified as the amount of histamine released as a percentage of the total histamine content (118). *Flow cytometric assays* are based on unique surface markers, e.g. CD63 or CD203c, that are expressed on basophils and can be measured with flow cytometry (119). In a resting basophil, CD63 is located inside the cell granules. Upon activation, granules fuse with the cell membrane and CD63 will be exposed on the cell surface (120). CD203c, on the other hand, is constitutively expressed at low levels on the surface membrane in resting basophils and is quickly up-regulated upon cell activation (121). Activation of basophils is shown in Figure 7.

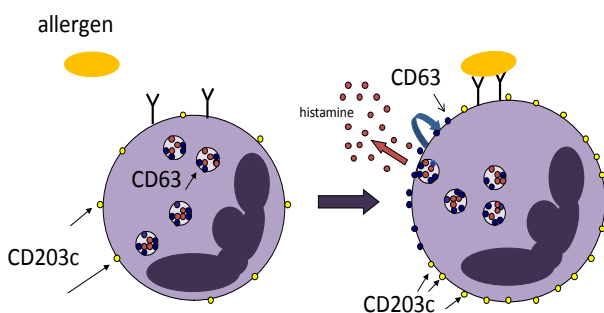


Figure 7. Activation of basophils.

In resting sensitized basophils, CD203c is expressed on the surface together with IgE-antibodies bound to the FcεRI receptor. Activation occurs after cross-linking of FcεRI-IgE-ab with the allergen and results in degranulation and release of mediators, e.g. histamine. CD203c is further up-regulated and CD63 will be expressed on the cell surface.

Depending on how basophils are stimulated by an allergen, basophil reactivity vs. basophil sensitivity will be measured (119). Basophil reactivity tests measure the maximal response of the basophils at one allergen concentration while basophil sensitivity tests investigate the basophils' allergen sensitivity. An important aspect in analyzing basophil sensitivity is the allergen dose-response curve. The dose-response curve of IgE-mediated responses in human basophils needs several 10-fold dilutions since there is a large variability of basophil sensitivity and responsiveness to the same allergen in different individuals (122).

There is one study reporting that 10-20% of the human population are non-responders, e.g. their basophils do not respond upon activation (123). Our clinical experience indicates a lower figure of approximately 5-10 % (124). However, in individuals with non-reacting basophils it is not possible to use any basophil activation test.

The method used in this thesis is basophil allergen threshold sensitivity test (CD-sens) (15). CD-sens is a functional *in vitro* test that determines the allergen threshold sensitivity, i.e. the lowest allergen dose that gives a 50% CD63 activation of the basophils. Thus activation of basophils at lower concentrations corresponds to high allergen sensitivity. Clinical research studies have shown good correlation between CD-sens, SPT, IgE-ab levels and allergic rhinitis. Studies investigating allergen sensitivity of patients with allergic asthma and showed also a good correlation with CD-sens (15-18).

3 OBJECTIVES

3.1 GENERAL OBJECTIVES

The general objective of this thesis was to evaluate children IgE-sensitized to peanut with a suspected IgE-mediated peanut allergy both clinically and immunologically with different diagnostic methods.

3.2 SPECIFIC OBJECTIVES

The specific objectives were:

- To evaluate CD-sens to peanut and Ara h 2 in relation to the outcome of DBPCFC in peanut sensitized children.
- To investigate if concentrations of IgE-ab to peanut, Ara h 1, Ara h 2, Ara h 3, Ara h 8 and Ara h 9 can predict the outcome of a DBPCFC.
- To study the reproducibility of oral food challenge regarding severity of reactions and eliciting dose and compare the reproducibility with the CD-sens method.
- To evaluate if concentrations of IgG₄-antibodies to peanut, Ara h 1, Ara h 2, Ara h 3 and Ara h 8 correlate to the outcome of oral peanut challenges.
- To investigate CD-sens to peanut and Ara h 8 in relation to oral peanut challenge in children IgE-sensitized to peanut, birch and Ara h 8.

4 MATERIAL AND METHODS

4.1 STUDY POPULATION AND STUDY DESIGN

Study subjects from two different populations are included in this thesis. The two first papers (I and II) are based on subjects from Sach's Children and Youth Hospital, Stockholm, Sweden (study population 1). Paper III is a case report of a child from study population 1. The fourth paper is based on subjects both from Sach's Children and Youth Hospital and from the 8-year follow-up of the BAMSE Cohort (study population 2). Paper V includes patients from study population 2. The number of patients and inclusion/exclusion criteria in the different studies/papers are presented in Figure 8.

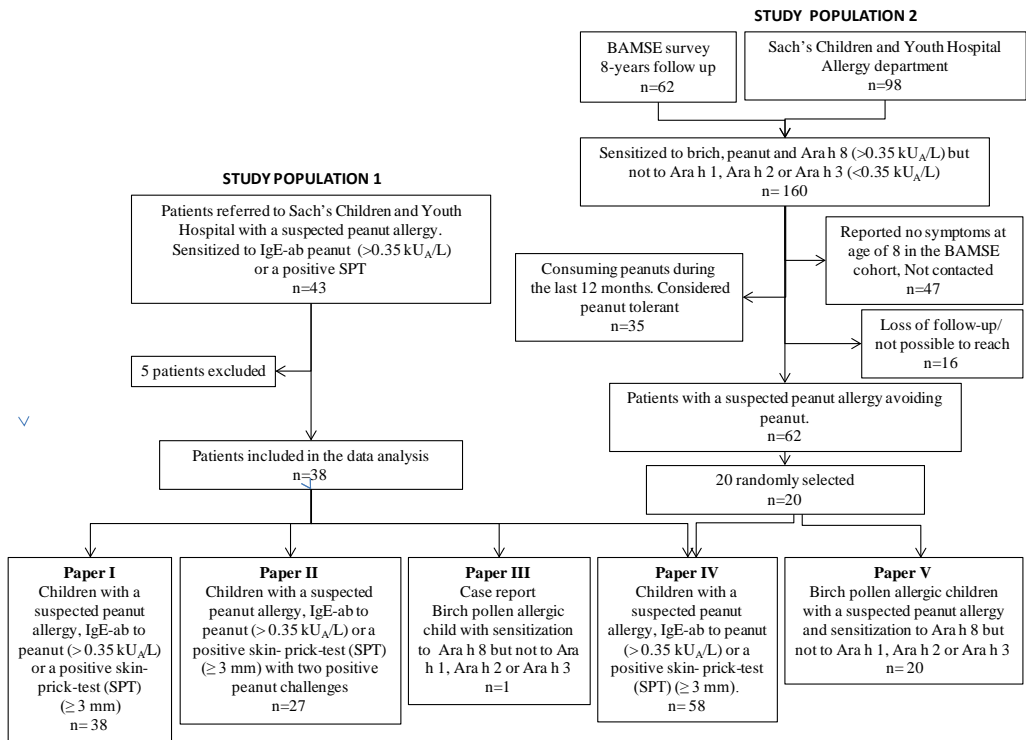


Figure 8. Study population 1 and 2 in relation to the five different papers in the thesis.

Sach's Children and Youth Hospital is one of two children's hospital in the Stockholm area. The Allergy department at the hospital has a food allergy unit that diagnoses and treats children with moderate to severe food allergy.

The BAMSE survey (n=4089) is an on-going population based birth cohort from predefined areas in Stockholm aiming at examining risk factors for allergy related diseases in childhood (125).

4.1.1 Study population 1

Forty-three children, aged 4-19 years, were invited to participate. The children were referred to the Allergy Department, Sach's Children and Youth Hospital due to a suspected peanut allergy.

Inclusion criteria were:

- IgE-ab to peanut ($> 0.35 \text{ kU}_A/\text{L}$) and/or a positive SPT to peanut ($>3\text{mm}$).
- Avoidance of peanuts for at least four weeks prior to inclusion.

Exclusion criteria were:

- History of anaphylaxis grade II-III to peanut confirmed in medical records.

Figure 9 describes the study design used with study population 1.

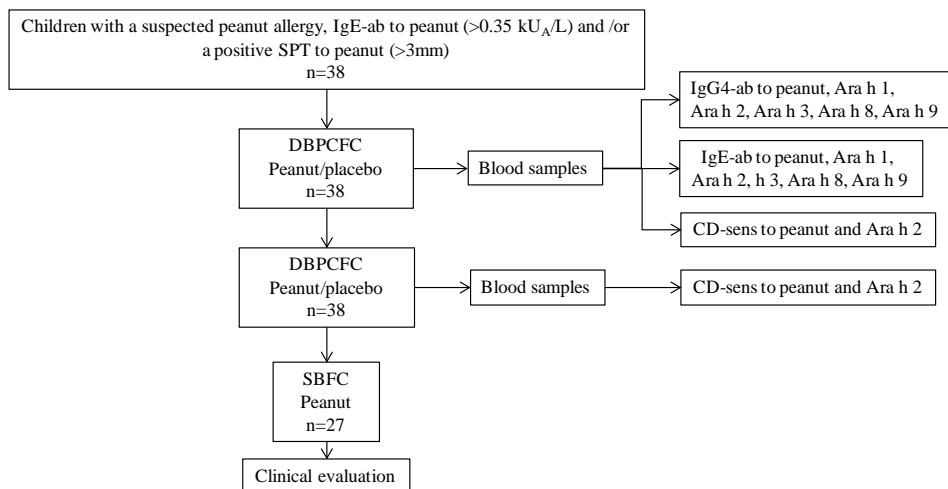


Figure 9. Study design study population 1.

Five children were excluded from the data analysis (Paper I, II and IV). One child did not complete the study, two other children did not follow the study protocol and the last two were found not to fulfill the inclusion criteria. Up to three challenges were performed, the first two were either placebo or active in random order and the third challenge was always active for ethical reasons. Venous blood samples were drawn before the first and second challenge for blood analysis.

4.1.2 Study population 2

Twenty children, 5-18 years, IgE-sensitized to birch and peanut, with a suspected peanut allergy were included. All children were part of another larger study at the Allergy Department, Sach's Children and Youth Hospital (n=160) (126). The children in the present study were randomly selected by inviting the first two study subjects each week that came to the clinic for an oral peanut challenge. All patients were IgE-sensitized to birch, peanut and Ara h 8 (> 0.35 kU_A/L) but not to Ara h 1, Ara h 2 and Ara h 3 (< 0.35 kU_A/L). Children were not included if they had a history of anaphylaxis grade II-III to peanut confirmed in medical records. Figure 10 describes the study design used with study population 2.

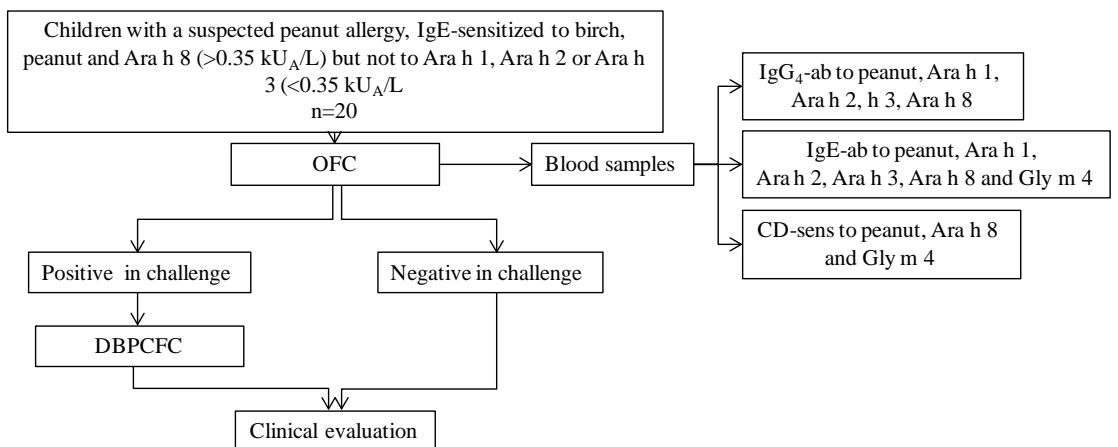


Figure 10. Study design study population 2.

Since only two of the children in study population 2 were recruited from the 8-year follow-up of the BAMSE cohort, this birth cohort will not be further discussed in this thesis. Information about the BAMSE cohort can be found elsewhere (125). An OFC to peanut was performed and if objective symptoms occurred a DBPCFC was scheduled. All blood samples were drawn before the OFC.

4.2 STUDY METHODS

4.2.1 Study subject characteristics

Medical records

Paper I, II, IV and V: Doctors' diagnosis of asthma, hay fever, eczema and food allergy was based on the International Classification of Disease, tenth revision (ICD-10) and collected from medical records.

In children reporting previous reactions to peanut, medical records were also used to collect information and evaluate the severity of the allergic reaction.

Paper III: After permission from the patient and her parents', information about the clinical history and laboratory results was collected from the patient's medical record at Sach's Children and Youth Hospital and from the Emergency clinic at Södertälje Hospital.

Clinical investigations

Paper I, II, IV and V: A medical history with focus on peanut allergy and medications was taken before the peanut challenges. Heart and lung auscultation, blood pressure measurements, and inspection of the oral cavity and skin were done before and during the challenges.

Telephone interview

Paper III: The patient's mother was interviewed over the telephone to collect more information about the patient's systemic reaction to peanut.

4.2.2 IgE-antibodies and IgG₄-antibodies

During the time-span of these research projects, laboratory techniques have developed and the Lower Limit of Quantification (LLOQ) for IgE-ab analysis has decreased from 0.35 kU_A/L to 0.1 kU_A/L. Therefore an IgE-ab level >0.35 kU_A/L was used at the inclusion to define a positive test.

Paper I, III-V: Circulating IgE and IgE-ab to birch (*Betula verrucosa*), peanut (*Arachis hypogaea*), Ara h 1, Ara h 2, Ara h 3, Ara h 8, Ara h 9, Bet v 1 and Gly m 4 were measured in serum with the ImmunoCAP® test (Phadia, Uppsala, Sweden). A positive test was defined as an IgE-ab level >0.1 kU_A/L.

Paper I: IgE-ab as a percentage of total IgE was calculated and designated "IgE-ab fraction".

Paper III: The recombinant 2 S albumin IgE-ab to Ara h 6 (sequence Acc. No. Q647G9) was analyzed at Thermo Fisher Scientific Diagnostic, Uppsala, Sweden, using an experimental ImmunoCAP® test (127). A positive test was defined as an IgE-ab level >0.1 kU_A/L.

Paper IV: IgG₄-ab to Ara h 1, Ara h 2, Ara h 3 and Ara h 8 in serum were measured with the ImmunoCAP® test. A positive test was defined as an IgG₄-ab level >0.07 mg/L.

4.2.3 CD-sens method

Blood samples were stored at +4°C for a maximum of 24 hours before cell analyses. Basophils from whole blood are stimulated with decreasing concentrations of an allergen. The basophils were stimulated with decreasing concentrations of desalted roasted peanut extract (final concentration 2.5-2500

ng/ml) recombinant Ara h 8 (final concentration 0.05-500 ng/mL) and Gly m 4 (final concentration 0.05-500 ng/mL) (Thermo Fisher Scientific, Uppsala, Sweden). Anti-FcεRI [IgE-dependent pathway] (Bühlmann Laboratories AG, Schönenbuch, Switzerland) and N-formyl-methionyl-leucyl-phenylalanin, fMLP [IgE-independent pathway] (Sigma Chemical Co, St. Louis, MO, USA) are used as positive controls and stimulated basophils are stained for CD63 and CD203c expression (Immunotech, Marseille, France). Cell surface expression of CD203c is used for identification of basophils and CD63 for detection of activated basophils. The basophils are finally analyzed in a Navios flow cytometer (Beckman Coulter, Inc., Fullerton, CA, USA). A detailed description of the CD-sens method is found in Figure 11.

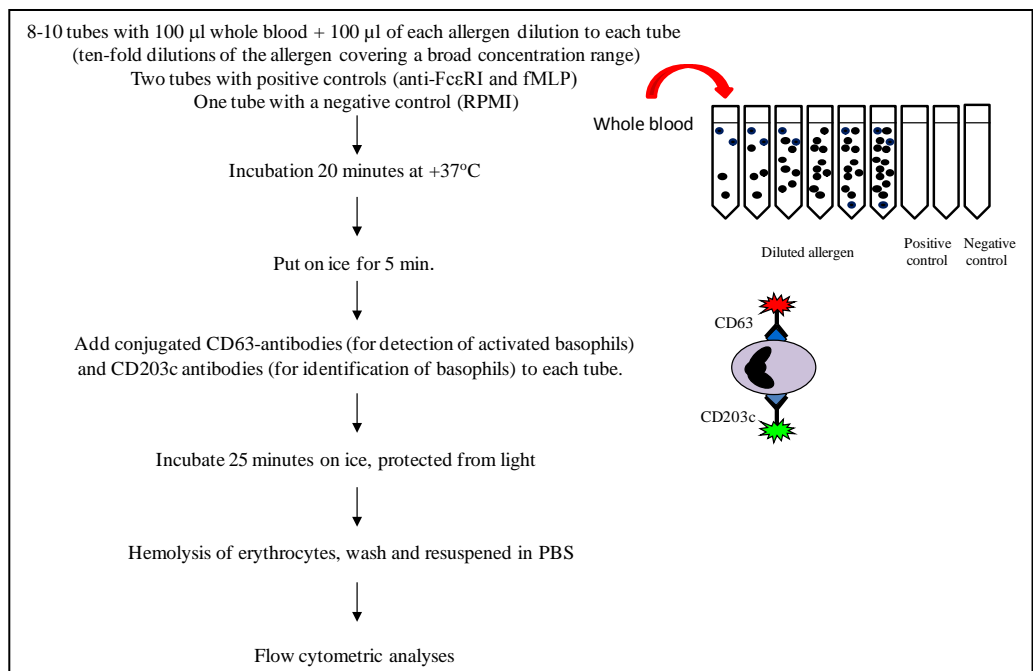


Figure 11. CD-sens method. Small volumes of blood (100 µl) are incubated at +37°C for 20 min with several dilutions of an allergen. An antibody is used as positive control. A phycoerythrin (PE) conjugated anti-CD203c mAb is used for identification of basophils and a fluorescein isothiocyanate (FITC) conjugated anti-CD63 mAb is used for detection of basophil activation. Following allergen stimulation the two conjugated antibodies are added and incubated for 25 min at +4°C. This is followed by lysis of the erythrocytes. The remaining leukocytes are washed and re-suspended in phosphate buffered saline (PBS). Surface CD203 and CD63 expression is measured by two-color flow cytometry.

The cut-off determining a positive test was set to 5%, i.e. twice the background. Individuals whose basophils responded with 0-5% CD63-upregulation after stimulation with anti-FcεRI, i.e. the positive control, are regarded as non-responders (14, 120). For individuals with a response between 5-16 % (low responders) the results should be interpreted with caution. The cut off 16% was calculated (mean 76% -3SD) from the positive controls of an in-house reference material from 264 allergic children and adults. The result from a CD-sens analysis is presented as a dose response curve as shown in Figure 12. CD-sens is measured as the lowest allergen concentration causing 50% of the maximum up-regulation of CD63 (LC50) and is defined as the inverted value of LC50 multiplied by 100 [$100(1/LC50)$]. A high CD-sens indicates a high basophil allergen threshold sensitivity (15).

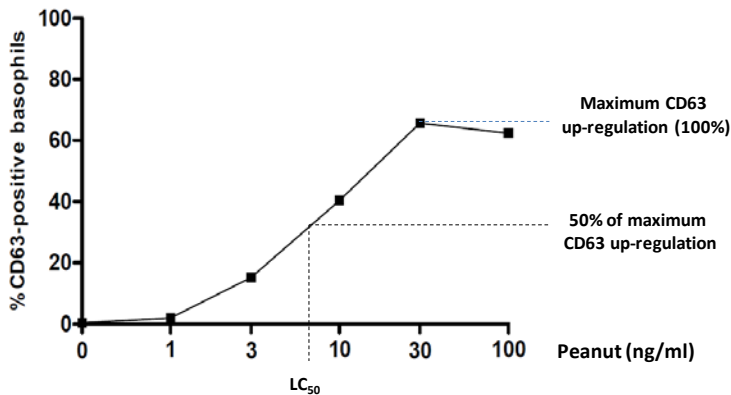


Figure 12. Dose-response curve. A dose-response curve from a CD-sens analysis in the study showing increased expression of CD63 with increased allergen concentrations up to maximum CD63 up-regulation.

Paper I-II: CD-sens to peanut and Ara h 2 were analyzed in all 38 children who participated in study population 1.

Paper III: CD-sens peanut and Ara h 8 were analyzed in the girl who participated in the case report.

Paper V: CD-sens to peanut, Ara h 8 and Gly m 4 were analyzed in all 20 children who participated in study population 2.

4.3 ORAL PEANUT CHALLENGES

The children had to be healthy at time of the challenge and no challenge was performed if the child had an on-going infection or an allergic reaction to other food or inhalant allergens. The challenge was also postponed if the child had used antihistamine less than four days or steroids less than two weeks prior to the challenge. Before challenge, medical treatment was prepared and a peripheral venous catheter was provided after local anesthesia (EMLA[®]).

Study population 1 (Paper I-III, IV)

A DBPCFC to peanut was performed, followed by a SBFC to peanut. The study design is shown in Figure 9. All three challenges were planned to be performed within a month and at least one week was allowed to go by between the challenges to avoid carry-over effects. For ethical reasons, children did not perform the SBFC if they had a very severe reaction and/or experienced a lot of discomfort at the DBPCFC. All challenges were performed using a validated recipe for peanut challenge medium (chocolate balls) containing 11% roasted peanuts and 7% fat (128). The challenge medium was given in increasing doses in 5 steps from 1 mg to 5 g (1 mg, 10 mg, 100 mg, 1 g and 5 g) every 30 minutes; 6.1 g is equivalent to approximately 8-9 whole roasted peanuts. A positive test was defined by objective symptoms and the severity of the reaction was graded from 1 to 5 according to Astier et al. (129) as shown in Table 3.

Table 3. Severity scoring according to Astier (129).

Symptom score	Symptoms
0	no symptoms
1	abdominal pain that resolved without medical treatment, rhinoconjunctivitis or urticaria <10 papules, rash
2	one organ involved *abdominal pain requiring treatment *generalized urticaria *non laryngeal angioedema *mild asthma (cough, fall of peak expiratory flow <20%)
3	two organs involved (of symptoms mentioned under 2)
4	three organs involved (of symptoms mentioned under 2) or asthma requiring treatment or laryngeal edema, or hypotension
5	cardiac and respiratory symptoms requiring hospitalization in the intensive care unit

A negative test was defined as no objective allergic symptoms within two hours after the challenge was completed.

A dietitian without knowledge of the study subjects prepared the challenge medium (chocolate balls) -two with peanut (active) and one without peanut (placebo) for every study subject. The chocolate balls were labeled, with the patient number and in random A and B for each patient, stored in a fridge and taken out in random order before each challenge. The third chocolate ball labeled C was always active and was used for the SBFC. The random code was concealed to the study subject, parents, nurses and the doctor during the two first challenges. The third challenge, when performed, was always done with a chocolate ball containing peanuts. This was known to the doctor and the nurses performing the challenges but not to the child or the parents.

Study population 2 (Paper IV-V)

An OFC to peanut was performed in all children. If an objective reaction occurred during the OFC, a DBPCFC was planned. The study design is shown in Figure 10.

The OFC was performed outside the pollen season; pure roasted peanut was given every 20 minutes in four steps from 100 mg to 5 g (100 mg, 1 g, 5 g and an additional 5 g). The total amount of peanut, 11.1 g corresponds to approximately 15-16 whole roasted peanuts. A negative challenge was defined as no objective allergic symptoms during the first hour after the challenge was completed. OAS, i.e. local symptoms from the oral cavity without symptoms from the skin, gastrointestinal tract, breathing difficulties or tissue swelling (68), was regarded as a negative outcome if the symptoms disappeared spontaneously without medication. A challenge was defined as positive if objective symptoms from the skin, gastrointestinal tract, respiratory tract and/or cardiovascular system occurred.

4.1 STATISTICAL ANALYSIS

The statistical analyses were performed using IBM SPSS v20.0, v22.0 (Chicago, IL, USA) and SAS v9.1 (SAS Institute Inc., Cary, NC, USA). The distributions of the variables from the two study populations were not normal distributed. Apart from skewed distributions there were values outside the measuring ranges and scores measured on ordinal scale. Therefore non-parametric statistical methods were used. No adjustment for multiple testing has been performed. Thus, significant results should be regarded as descriptive and explorative.

4.1.1 Wilcoxon rank-sum test (I, IV, V)

In paper I and IV Wilcoxon rank sum test was used to compare differences in IgE-ab, IgG₄-ab, CD-sens, eliciting dose and severity scoring in study subjects positive or negative in peanut challenges. In Paper V the same test was used to assess differences of CD-sens and IgE-ab in children with or without OAS.

4.1.2 Spearman rank-order correlation (I, II, IV)

Spearman rank-order correlation (r_s) was used to assess the relation between the cumulative amount of peanut tolerated before reaction at challenge and the severity of the reaction, in paper I. Correlations between the first and second value of severity score, eliciting dose and CD-sens were also estimated with Spearman rank-order correlation in Paper II. In Paper IV correlations were calculated between IgE-ab, IgG₄-ab and the ratio of IgE/IgG₄-ab.

4.1.3 Wilcoxon signed-rank test (II)

Wilcoxon signed-rank test was used to test differences between the first and the second challenge for severity score, eliciting dose and CD-sens value in study subjects who reacted to both active challenges. The value for eliciting dose and the CD-sens value was tested on log-transformed values, thus testing the relative change.

4.1.4 **Bland-Altman plot (II)**

Bland-Altman plots were used to present the differences in severity score, eliciting dose and CD-sens value from the first and second active challenge in Paper II.

4.2 **ETHICAL APPROVAL**

The studies in the thesis were approved Regional Ethical Review Board at Karolinska Institutet in Stockholm, Sweden. (Identification number 2008-1001-31/2 and 2010/133-31/3). Written informed consent was obtained from all study subjects and their parents before the children were included in the study.

5 RESULTS

5.1 PAPER I

Paper I demonstrates that CD-sens and CRD are useful tools for diagnosis of peanut allergy.

5.1.1 DBPCFC to peanut

Thirty-eight children were evaluated and no child reacted to placebo. Twenty-five children (66%) reacted at challenge while thirteen children (34%) passed without any objective symptoms. Ten children with a positive and eight with a negative peanut challenge claimed they had never eaten peanuts. Five children with negative challenges reported previous allergic reactions to peanut. The symptoms were scored according to Astier et al (129), shown in Table 3, and the severity of the reaction was mild to moderate (Grade 1-3) in fifteen children (60%). Nine children (36%) had severe reactions and one child (4%) was sent to the intensive care unit for observation (Grade 5) (Figure 13).

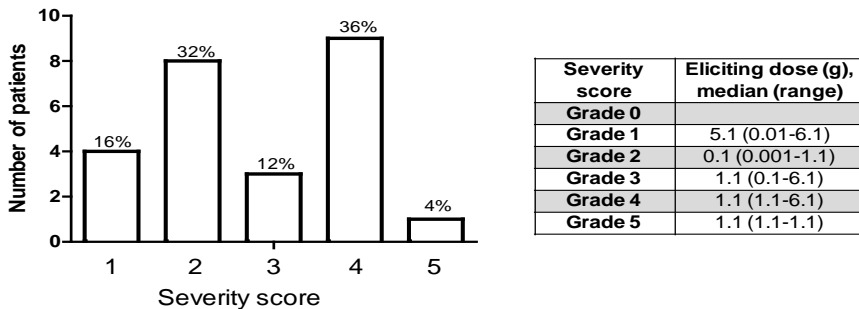


Figure 13. Severity score and eliciting dose in children positive in challenge from study population 1.

There was no association between the eliciting dose of peanut at the challenge and the severity of the reactions ($r_s=0.21$, $p=0.32$), which means that some children reacted to low doses with severe symptoms while other children were able to eat more peanut without severe symptoms.

5.1.2 CD-sens to peanut and Ara h 2

Ninety-two percent (22 /24) of the children with a positive DBPCFC were positive in CD-sens to peanut and ninety-two percent (23/25) were positive to Ara h 2. Two children were classified as low responders, having too weak response to the positive control, anti-FcεRI (<16%) to allow further evaluation. Of the thirteen children with a negative DBPCFC 77% (10/13) were negative in CD-sens after stimulation with peanut and Ara h 2 as shown in Figure 14.

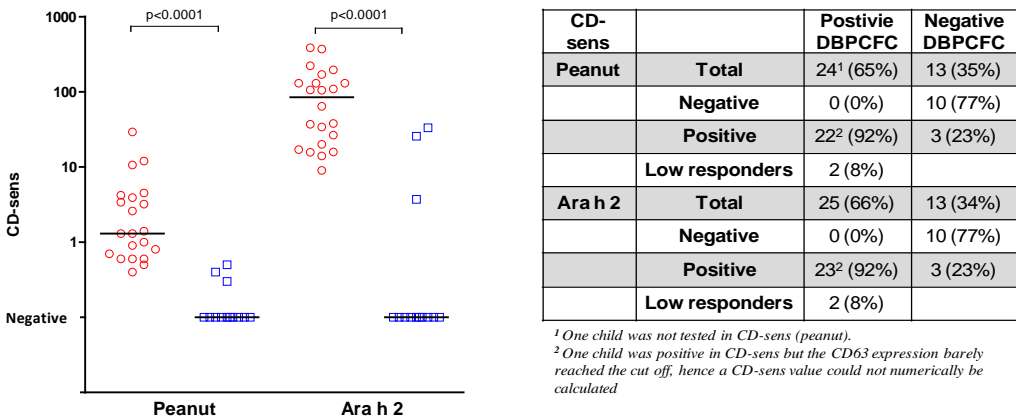


Figure 14. CD-sens to peanut in children with a positive (○) or negative (□) DBPCFC. Horizontal bars, median.

Three children negative in challenge had positive values in CD-sens to peanut and Ara h 2. All of these three had low concentrations of IgE-ab to Ara h 2 (0.2, 0.9 and 1.9 kU_A/L). However, all children negative in CD-sens to peanut were negative in DBPCFC as shown in Figure 14. Children with a positive DBPCFC had significantly higher levels of peanut CD-sens, [1.3 (range 0.4-29.3)] than children with negative DBPCFC [0 (range 0-0.5)] (p<0.0001). The value for CD-sens to Ara h 2 differed also significantly between children positive, 84.5 (range 9.0-385.0) and negative 0 (range 0-33.2) in DBPCFC (p<0.0001). One child was positive in CD-sens but the CD63 expression barely reached the 5% cut off, therefore a CD-sens value could not be calculated.

5.1.3 IgE-ab to peanut and peanut components

Children with a positive DBPCFC had significantly higher median concentrations of IgE-ab to peanut, Ara h 1, Ara h 2 and Ara h 3 compared to those negative at the challenge ($p < 0.0001$ for all). No significant differences were found for the median concentrations of IgE-ab to Ara h 8 and Ara h 9 between children positive vs. negative in DBPCFC (Figure 15). Two children positive in DBPCFC had low IgE-ab concentrations (0.6 and < 0.1 kU_A/L) to Ara h 2. Three children negative in DBPCFC had IgE-ab to Ara h 2 (0.2; 0.9; 1.9 kU_A/L).

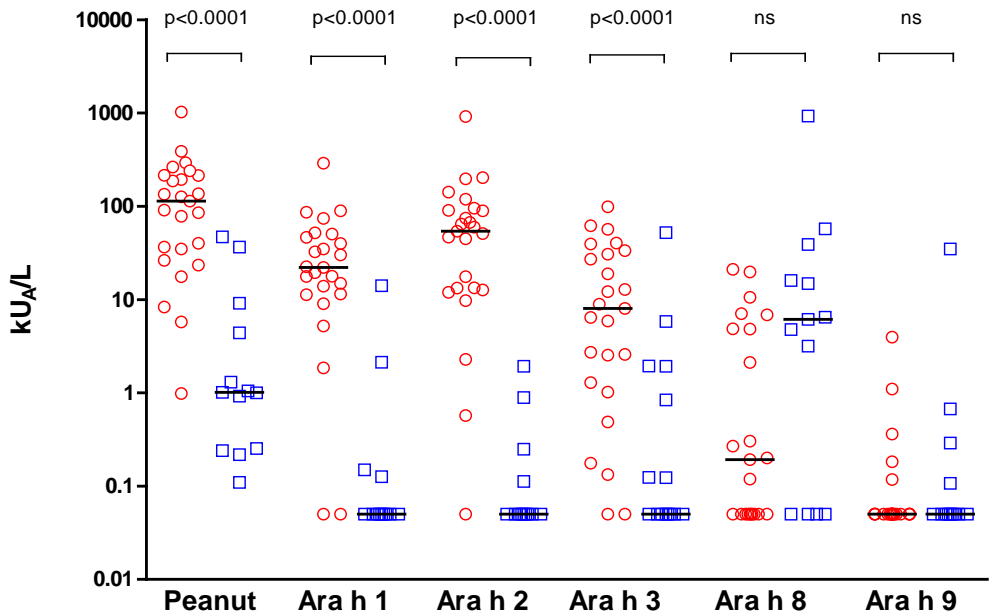


Figure 15. IgE-ab to peanut and peanut components in children with a positive (○) or negative (□) DBPCFC. Horizontal bars = median, ns= not significant

The IgE-ab fraction was calculated and children positive in DBPCFC had a median IgE-ab fraction of 26% (range 1-72%) for peanut and 11% (range 0-55%) for Ara h 2, while those negative in DBPCFC had 0% (range 0-10%) and 0% (range 0-2%) $p < 0.0001$ for both.

5.1.4 Accuracy of peanut allergy diagnosis

All children in study population 1 had a suspected peanut allergy and had previously been recommended by their doctor to avoid peanuts. Of the 38 children included, 53% (20/38) had a convincing history of peanut allergy. However, 47% (18/38) children had been diagnosed via laboratory result showing IgE-sensitization to peanut although they claimed that they had never eaten peanuts. Forty percent (10/25) of peanut allergic children (positive in peanut challenge) and sixty-two percent (8/13) of the tolerant children (negative in challenge) had been diagnosed by clinical work-up without a previous reaction. Adrenaline auto-injector had been prescribed before challenge to 76% (19/25) of the children positive and 85% (11/13) of the children negative in challenge (Figure 16).

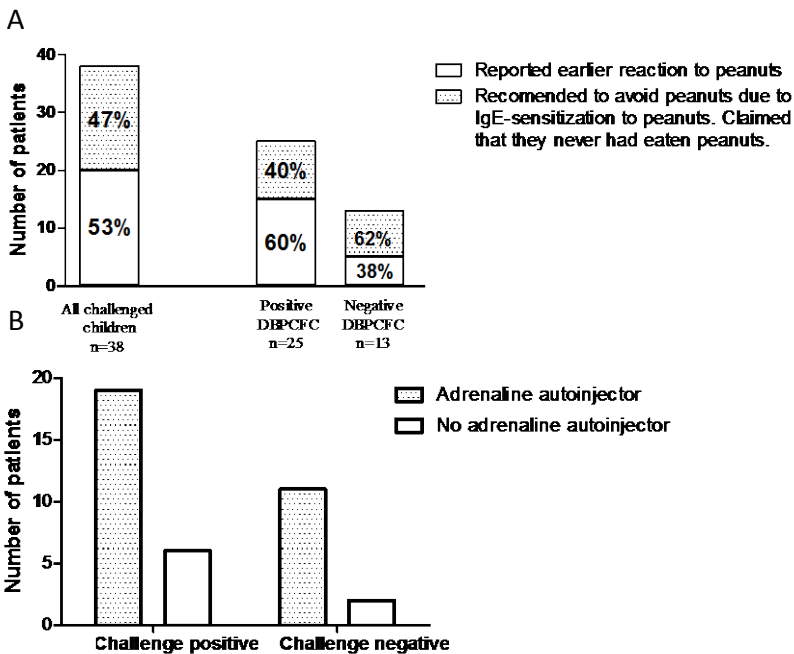


Figure 16.
A. History of clinical reaction at inclusion and reaction in DBPCFC.
B. Prescribed adrenaline auto-injector before inclusion in the study.

5.2 PAPER II

Paper II concludes that the reproducibility of a positive or negative test result is 100% for both CD-sens and peanut challenge. However, severity score and eliciting dose were not reproducible for oral peanut challenges when the challenge was repeated.

5.2.1 Reproducibility of oral peanut challenges

Of the 38 children in study population 1, 27 were performing three challenges (two active and one placebo). Eleven children were excluded since they reacted severely or experienced a lot of discomfort at the first DBPCFC. Fourteen children (52%) reacted at both peanut challenges, but not to placebo and were considered allergic to peanut. The severity of the challenge reactions was scored and the eliciting doses were determined in every patient. Three children (J28, J36, J41) reacted at the same severity score and had the same eliciting dose at the two peanut challenges. All other children (n=11) scored differently or reacted at different eliciting doses. (Figure 17 and Table 4).

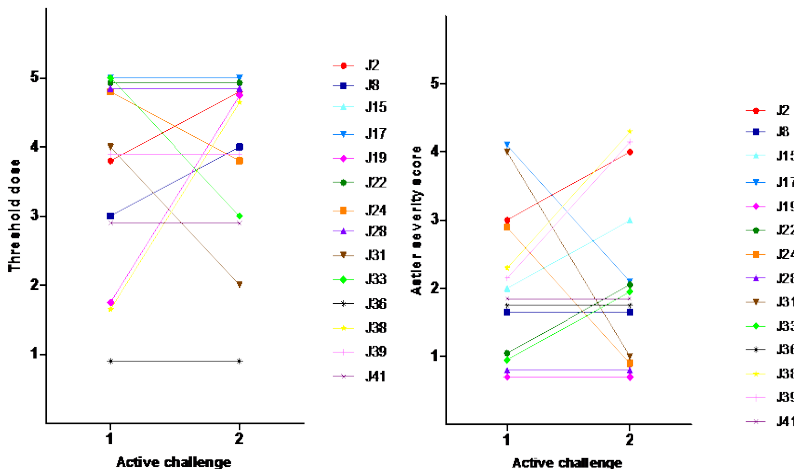


Figure 17. Eliciting dose and severity scoring in the same peanut allergic child at the two peanut challenges. The amount of peanut was the same for each child at the five dose steps: Dose 1=0.01 g; Dose 2 = 0.01 g; Dose 3=0.1 g; Dose 4=1 g; Dose 5=3.6-5 g

Table 4. Age, sex and challenge outcome in peanut allergic children.

Patient	sex	Age (years)	1 st peanut challenge		2 nd peanut challenge			
			Symptoms	Severity score	peanut (g)	Symptoms	Severity score	Peanut (g)
J2 ◆	female	12.7	urticaria, stomach-ache, mouth-itch, tiredness	3	1.1	asthma, skin-itch, rhinitis, urticaria, stomach-ache	4	6.1
J8 ■	male	15.4	stomach-ache, mouth-itch	2	0.1	conjunctivitis, rhinitis, stomach-ache	2	1.1
J15 ▲	female	17.4	stomach-ache, mouth-itch	2	0.1	mouth-itch, stomach-ache, urticaria, rhinitis	3	1.1
J17 ▼	female	14.0	tiredness, mild asthma, mouth-itch	4	6.1	tiredness, stomach-ache, vomiting, conjunctivitis	2	6.1
J19 ◆	male	9.8	mouth-itch	1	0.01	mouth-itch, stomach-ache	1	3.6
J22 ◆	male	5.4	stomach-ache	1	4.1	conjunctivitis, stomach-ache, tiredness	2	6.1
J24 ■	female	17.6	tiredness, vomiting, mouth-itch, stomach-ache, urticaria	3	6.1	mouth-itch	1	1.1
J28 ▲	male	7.6	stomach-ache	1	6.1	erythema, stomach-ache, tiredness	1	6.1
J31 ▼	male	19.0	mouth-itch, urticaria, cough, stomach-ache, tiredness, erythema	4	1.1	mouth-itch	1	0.01
J33 ◆	female	12.2	mouth-itch, tiredness, stomach-ache	1	6.1	stomach-ache, tiredness	2	0.1
J36 ✱	male	18.4	mouth-itch, stomach-ache, tiredness	2	0.001	mouth-itch, stomach-ache	2	0.001
J38 ▲	female	7.9	cough, tiredness	2	0.01	hoarseness, cough, mouth-itch, conjunctivitis, rhinitis, vomiting, stomach-ache, tiredness, urticaria	4	6.1
J39 +	male	5.9	mouth-itch, stomach-ache, tiredness	2	1.1	mouth-itch, asthma	4	1.1
J41 ✱	female	11.8	mouth-itch, stomach-ache, tiredness	2	0.1	mouth-itch, stomach-ache, tiredness	2	0.1

Thirteen children (48%) did not react at any of the three challenges. All children negative at the challenges consumed full doses (6.1 g peanut). In children with a positive challenge the arithmetic mean of difference in the severity scores (challenge 2 - challenge 1) was 0.143 (p=ns) and the geometric mean of the ratio of the doses (challenge 2/challenge 1) was 1.834 (p=ns). No association was

obtained between the first and second challenge regarding severity score ($r_s=0.11$; $p=ns$) and eliciting dose ($r_s=0.35$; $p=ns$).

5.2.2 Reproducibility of CD-sens to peanut

CD-sens was performed with blood drawn before the first and second challenges in all children ($n=26$) except one. In this child, blood was collected at the second and third challenges due to a misunderstanding. All children with a positive CD-sens at the first challenge were also CD-sens positive at the second challenge. Twelve of fourteen children with a positive challenge were also positive in CD-sens to peanut (Figure 18). The remaining two could not be evaluated since they were low responders i.e. had too weak response to the positive control anti-FcεRI ($<16\%$). Three children had slightly positive values in CD-sens (0.3-0.5) but were negative in challenge. However, all ten children negative in CD-sens were also negative at both peanut challenges. The geometric mean of the ratio of CD-sens values (challenge 2 /challenge 1) in children with a positive challenge was 1.035 ($p=ns$) and the association between the two CD-sens values was strong ($r_s=0.94$, $P<0.001$).

Patient	1 st challenge		2 nd challenge	
	CD-sens Peanut	LCS0 (AU/mL)	CD-sens Peanut	LCS0 (AU/mL)
J2	4.2	23.6	4.8	21.1
J8	1.3	77.2	1.3	74.5
J15	1.6	62.4	1.3	78.4
J17	1.4	69.4	1.8	56.0
J19	14.8	6.8	12	8.3
J22	Low responder	Low responder	Low responder	Low responder
J24	0.7	149.7	0.6	166.4
J28	Low responder	Low responder	Low responder	Low responder
J31	192	0.5	188	0.52
J33	0.4	263.0	0.5	220.0
J36	19.2	5.2	10.6	9.4
J38	0.4	272.0	1.0	96.0
J39	0.6	162.0	0.7	136.0
J41	2.4	41.7	3.9	25.5

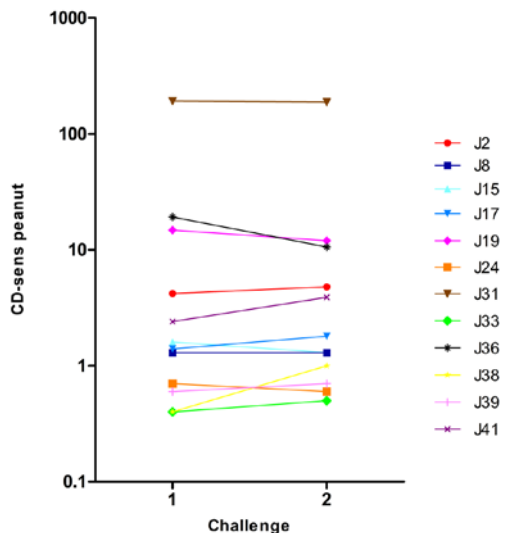


Figure 18. CD-sens to peanut in the same peanut allergic child before the two first challenges.

5.3 PAPER III

Paper III and V show that CD-sens to Ara h 8 is positive in a majority of the children IgE-sensitized to birch and Ara h 8 and indicate that IgE-sensitized basophils can be activated by an intact Ara h 8 allergen and initiate allergic inflammation.

5.3.1 Birch pollen allergy and cross-sensitization to peanut

Paper III is a case report describing a 16-year-old girl with rhinoconjunctivitis during birch pollen season, who also experiences OAS when eating some fruits. She passed two oral peanut challenges (study population 1) and started to eat peanuts at home without objective symptoms. However, on one occasion she ate a large amount of peanuts (approximately 300 g), developed a severe allergic reaction and needed acute medical treatment.

At time of challenge she had IgE-ab to peanut and Ara h 8 but not to Ara h 1, Ara h 2 or Ara h 3. CD-sens to peanut was also negative. After the acute reaction when eating 300 g of peanuts she was re-evaluated for a suspected peanut allergy. Interestingly, the IgE-ab pattern to the various peanut allergen components was unchanged, which was also the case when the analyses were

repeated in 2013. CD-sens was also re-analyzed in 2013, and was still zero to peanut but positive to Ara h 8, as shown in Table 5. The girl is still eating peanuts, approximately a handful at a time (~ 40 g), but avoids larger amounts.

Table 5. Immunological analysis.

	2008	2011	2013
IgE [#]	297	n.t.	n.t.
IgE-ab Bet v 1*	22	n.t.	n.t.
IgE-ab peanut*	1.0	2.4	1.1
IgE-ab Ara h 1*	<0.1	<0.1	<0.1
IgE-ab Ara h 2*	<0.1	<0.1	<0.1
IgE-ab Ara h 3*	<0.1	<0.1	<0.1
IgE-ab Ara h 6*	<0.1	<0.1	n.t.
IgE-ab Ara h 8*	39.1	12.0	12.7
IgE-ab Ara h 9*	<0.1	<0.1	<0.1
CD-sens peanut	0	n.t.	0
CD-sens Ara h 8	n.t.	n.t.	12.5

5.4 PAPER IV

Paper IV shows that tolerant children with peanut sensitization are characterized by low levels of IgG₄-ab to peanut and Ara h 2 but relatively high IgG₄/IgE-antibody ratios.

5.4.1 IgG₄-antibodies and oral peanut challenges

Children from both study populations were included (study population 1 and 2). Of 58 investigated patients 25 were considered as positive (allergic) at the challenge and 33 as negative (tolerant).

Allergic children had low levels of IgG₄-ab to peanut [median: 0.28 (range: <0.07-10.7) mg/L] and Ara h 2 [0.15 (<0.07-2.3) mg/L] but nonetheless levels significantly higher than those in tolerant children [peanut: 0.14 (<0.07-0.83) mg/L and Ara h 2: <0.07 (<0.07-0.62) mg/L] $p<0.05$ and $p<0.001$, respectively. However, tolerant children had higher concentrations of IgG₄-ab to Ara h 8 [<0.07 (<0.07-2.2) mg/L] than allergic children [<0.07 (<0.07-0.56) mg/L; $p<0.05$] (Figure 19). The median levels of IgG₄-ab to Ara h 1 and Ara h 3 in tolerant children were <0.07 (<0.07-0.1) mg/L and <0.07 (<0.07-0.9) mg/L, respectively and in allergic children <0.07 (<0.07-0.37) mg/L and <0.07 (<0.07-0.35) mg/L, respectively. Statistical analyses to compare differences in Ara h 1 and 3 were not done, since most IgG₄-ab concentrations were below LLOQ in both allergic and tolerant children.

Significant correlations were found between concentrations of IgE-ab and IgG₄-ab to Ara h 2 and peanut in the allergic children ($r_s=0.54$; $p<0.01$ and $r_s=0.44$; $p<0.05$, respectively) but not for the other components or for the tolerant children. The peanut- and Ara h 2 IgG₄/IgE-antibody ratios in peanut tolerant individuals were significantly higher than in the allergic children ($p<0.05$ and $p<0.001$, respectively) (Figure 19). Most of the tolerant children had very low or undetectable IgG₄- or IgE-ab levels to Ara h 1 and 3, precluding antibody ratio

calculations. There was no significant difference in the Ara h 8 IgG₄-/IgE-ab ratio between allergic and tolerant children.

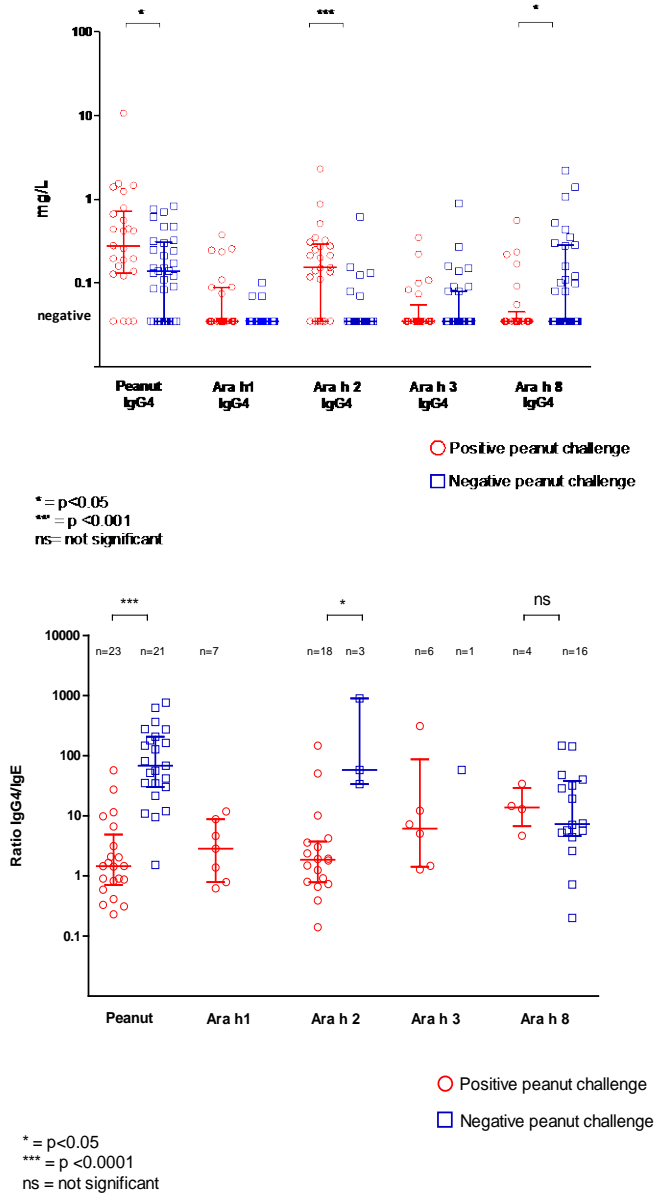


Figure 19. IgG₄ab and IgG₄ab /IgE-ab ratio to peanut and peanut components in children positive (o) or negative (□) in challenge. Median and interquartile range is included in the figure.

5.5 PAPER V

In paper V, none of the children sensitized to Ara h 8 but not to Ara h 1, Ara h 2 or Ara h 3, had a systemic allergic reaction at the challenge. However, the majority of children (85%) were positive in CD-sens to Ara h 8, indicating that they had Ara h 8 IgE-ab sensitized basophils which could be activated by intact Ara h 8 proteins and initiate an allergic inflammation.

5.5.1 CD-sens, Ara h 8 and oral peanut challenges

IgE-ab

At the time of challenge, all children had IgE-ab (>0.1 k_AU/L) to peanut and Ara h 8. The median (range) for peanut was 0.7 (0.1-16.1) k_AU/L, for Ara h 8, 6.4 (0.5-131.7) k_AU/L and for Bet v 1 30.1 (1.5-202.6) k_AU/L. Three children had low levels of IgE-ab to Ara h 2 (0.2-0.4) k_AU/L. All children but one had IgE-ab to Gly m 4 with a median of 4.9 (1.5-18.9) k_AU/L. There was no significant difference in IgE-ab levels to peanut ($p=0.93$) or Ara h 8 ($p=0.93$) in children with or without OAS at the challenge.

CD-sens

All children but one (Patient 4) were negative in CD-sens to peanut. This child had IgE-ab to Ara h 2 (0.4 k_AU/L) at the time of challenge, but not at inclusion. Seventeen children (85%) were positive in CD-sens to Ara h 8. At time of the challenge the median of CD-sens to Ara h 8 was 5.9 (0-82.8). Concentrations of IgE-ab to Ara h 8 in the three children with negative CD-sens to Ara h 8 were 0.5, 0.8 and 6.3 k_AU/L and the corresponding IgE-ab fraction size to Ara h 8 was 0.03%, 0.04% and 4.9%, respectively (Table 6).

There were no significant difference in CD-sens to Ara h 8 ($p=0.42$) between children with ($n=5$) and without ($n=15$) OAS at the peanut challenge.

Table 6. Immunological analysis and symptoms at challenge

Patient id	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Symptoms	0	0	0	0	OAS	OAS	OAS	0	0	0	OAS	0	OAS	0	0	0	0	0	0	0
CD-sens peanut	0	0	0	12.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CD-sens Ara h 8	1.5	0	23.2	82.8	5.9	0	38.9	6.0	6.1	30.6	10.4	4.8	0	1.2	4.0	68.7	13.5	3.0	2.0	50
CD-sens Gly m 4	1.1	Positive	0	51.7	0	0	5.0	2.0	0	8.2	41.8	14.6	0	1.2	0	15.4	0	0	0	17
IgE*	359	2460	1030	169	119	1110	959	1670	253	439	428	2080	130	54.6	264	44	59	147	503	190
IgE-ab Peanut**	3.1	0.7	4.9	2.9	0.6	0.3	16.1	5.7	1.8	0.7	4.8	5.4	0.3	0.3	0.6	0.2	0.13	0.2	0.6	1.0
IgE-ab Ara h 8**	6.4	0.8	44.9	38.1	6.4	0.5	16.4	132	1.4	11.1	14.9	21.7	6.3	2.4	7.4	4.5	2.9	1.1	5.1	17.6
IgE-ab Ara h 1**	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
IgE-ab Ara h 2**	0.1	0.2	0.1	0.4	<0.1	0.1	0.1	0.1	<0.1	<0.1	<0.1	0.3	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
IgE-ab Ara h 3**	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
IgE-ab Ara h 9**	0.2	0.1	0.1	0	0	0.1	4.3	0.2	0	0	0	0.1	0	0	0	0.3	0	0	0	0
IgE-ab Gly m 4**	7.5	0.2	21.0	14.9	1.7	0	37.2	77.6	1.1	16.2	19.7	25.6	5.4	2.0	4.5	1.9	1.4	0.5	3.9	15.8
IgE-ab Bet v 1**	47.1	1.5	124	97.3	35.1	25.1	36.4	203	3.6	76.6	48.8	135	21.1	8.4	20.6	9.8	12.6	7.0	18.7	52.0
% IgE Gly m 4	2.1	0.01	2.1	8.8	1.4	0.002	3.9	4.6	0.4	3.7	4.6	1.2	4.1	3.6	1.7	4.4	2.3	0.4	0.8	8.3
% IgE Ara h 8	1.8	0.03	4.4	22.5	5.3	0.04	1.7	7.9	0.6	2.5	3.5	1.0	4.9	4.3	2.8	10.2	4.9	0.7	1.0	9.3
% IgE Peanut	0.9	0.03	0.5	1.7	0.5	0.03	1.7	0.3	0.7	0.2	1.1	0.3	0.2	0.6	0.2	0.5	0.2	0.1	0.1	0.5
% IgE Bet v 1	13.1	0.1	12.1	57.5	29.5	2.3	3.8	12.1	1.4	17.5	11.4	6.5	16.2	15.3	7.8	22.2	21.4	4.8	3.7	27.3

OAS = Oral allergy syndrome

*kU/L

**kU_A/L

6 DISCUSSION

This thesis focuses on peanut allergy and the aim was to evaluate clinical and immunological characteristics through peanut challenges, CRD and CD-sens in children IgE-sensitized to peanut with a suspected peanut allergy.

6.1 ORAL PEANUT CHALLENGES

The main goal of a peanut challenge is to determine, under safe conditions, if a patient is allergic or not. However, severe reactions do happen (130), and even though DBPCFC is the gold standard for peanut allergy diagnosis, (99-103) reactions to placebo also occur (131) and have been reported in significant numbers (132, 133). Furthermore, performing DBPCFC is also difficult and the procedure poses a number of practical problems and the method has several pitfalls (134, 135).

In our study no child reacted to placebo and all the children who were positive in the first active challenge were also positive in the second active challenge, indicating that peanut challenge is reproducible for a positive or negative challenge outcome.

Several factors affect the outcome of a peanut challenge in addition to the amount of allergen given during challenge, for example gastric or respiratory infections, hormonal factors (menstruation), psychogenic factors (stress) and drugs (e.g. antihistamine, corticosteroids) (28, 136). A recently published study investigating challenge outcome in peanut allergic individuals could not find any consistent relationships between the severity of challenge outcome and the cumulative dose given (130). This is in line with the results of Paper I, showing that 66% of the children reacted to the peanut challenge but we were not able to see any association between the severity of the reaction and the amount of peanut eaten.

Several studies have also tried to determine risk factors and eliciting dose in peanut allergic patients to predict the clinical sensitivity (137-140). Skripak and colleagues evaluated the effect of 23 weeks of oral immunotherapy with milk by using DBPCFC before and after treatment (141). The outcome of the food challenge was based on the lowest dose that caused a reaction and not on changes in the severity of the reactions. In that study 29% of the patients in the placebo group (n=7) did not react at the same dose when the DBPCFC was repeated (141). Van der Zee and colleagues aimed to evaluate if presence of risk factors for a severe peanut allergy is associated with the patient's clinical sensitivity. To do this they determined the eliciting dose of peanut in a DBPCFC and examined whether this would predict the severity of the allergic reaction. However, they were not able to find a relation between the eliciting dose in DBPCFC and the severity of a reaction at home (137). This is in line with our results showing that the severity score and the eliciting dose were not reproducible in a repeated oral challenge and may indicate that a child with a mild reaction could react severely on renewed exposure to peanuts or vice versa.

A positive food challenge reaction should always be based on objective symptoms (99-103, 142). For ethical reasons it is not always possible to provoke objective symptoms in all patients, which is a problem when evaluating challenge outcome (136). This could also be demonstrated in Paper II. One girl (J33) experienced symptoms from the mouth cavity (6.1 g) at the first challenge but no medical treatment was needed. A few hours later she suffered a late reaction with severe stomach ache and fell asleep. At the second active challenge she reacted to a low dose (0.1 g) with severe stomach ache that required medical treatment and the challenge were stopped. Another child (J36) refused to eat more than the first dose (1 mg) because of mouth itch and stomach pain that resolved after medication at both active challenges. He passed the placebo challenge without any symptoms.

There are several guidelines for how to perform a DBPCFC (99, 100, 102, 103, 142). However, different research groups still use their own challenge schedules

and severity symptom scores (63, 129, 137, 140, 143-145). In 2012 a new consensus report for standardization of DBPCFC was published with practical recommendations for both challenge schedules and severity scoring, which hopefully will contribute to harmonizing the challenge procedure (101). Unfortunately, there is still no objective measurement combining both severity of the reaction and amount of peanut eaten at the challenge.

6.2 COMPONENT RESOLVED DIAGNOSTICS

There has been intense research worldwide concerning peanut allergy during the last decade. In the beginning of 2007, when our studies were planned, CRD was not yet in clinical use. Sensitization and the clinical importance of different peanut allergens were widely discussed. More research was needed investigating peanut components in relation to the gold standard, DBPCFC.

Peanut storage proteins

We found in Paper I that the IgE-ab levels to the storage proteins Ara h 1, Ara h 2, and Ara h 3 are significantly increased in children positive in DBPCFC to peanut compared to those negative in challenge. This result is in line with other reports investigating peanut allergy in relation storage proteins and Ara h 2 seems to be the most important allergen (111-113, 146-148). However, lack of IgE-ab to Ara h 2 does not exclude a peanut allergy and sensitization patterns vary in different regions worldwide (113, 149).

In Paper I, five patients negative at the peanut challenge had IgE-ab to the storage proteins (>0.1 kU_A/L). Three had IgE-ab to Ara h 2 (>0.1 - <2.0 kU_A/L), one child had IgE-ab Ara h 3 (5.8 kU_A/L) and the fifth child had IgE-ab to Ara h 1 and Ara h 3 (14.1 and 0.8 kU_A/L, respectively). Elevated levels of Ara h 2 in peanut tolerant children have also been observed by others (150). Cross-reactivity between the peanut and tree nut allergens has been described (105, 151) and could be a speculative explanation for the elevated levels of IgE-ab to the peanut storage proteins. However, the three children with IgE-ab to Ara h 2

but negative in challenge were also weakly positive in CD-sens to peanut, which may be a sign of developing tolerance or a developing peanut allergy. Of the children positive in challenge, all but one were positive for IgE-ab to Ara h 2. This child was negative in all components including IgE-ab to Ara h 2 but positive in IgE-ab to peanut. Unfortunately this child was a low responder in CD-sens and could not be evaluated.

There is currently no treatment for peanut allergy except avoidance. However, anti-IgE-ab treatment may be useful therapy in the future. An earlier study has shown that a small IgE-ab fraction, <1% clinically relevant IgE-ab of IgE, is important for successful anti-IgE treatment (152). In our study, the IgE-ab fraction differs significantly between children positive and negative in DBPCFC. Children positive in DBPCFC had significantly larger IgE-ab fractions (25% to peanut and 11% to Ara h 2) than those who were negative. This suggests that the fraction size should be considered before starting treatment with anti-IgE in patients with food allergy.

6.3 CD-SENS

Peanut and Ara h 2

In Paper I we concluded that peanut allergic children have significantly higher levels of CD-sens to peanut compared to tolerant children. CD-sens has earlier been shown to correlate with asthma sensitivity in both stable and instable asthma (16, 17). Recently published studies investigating tolerance to cow milk showed that children with a severe milk allergy had more activated basophils than children who tolerated milk. It was also possible to discriminate between children who tolerated heated milk and those who did not (153, 154). This is in line with our study showing that children who tolerated peanuts were negative in CD-sens to peanut. These investigations may indicate that CD-sens can signal presence or absence of tolerance and when CD-sens is negative the probability for an allergic reaction is low.

A recent published study investigating peanut allergic children and severity of the allergic reaction after accidental ingestion of peanut showed that children with anaphylaxis had significant higher levels of CD-sens to peanut than children without anaphylaxis (155). Paper II was planned to investigate the severity of the outcome of peanut challenge and compare the results to peanut CD-sens. This aim could not be achieved: the eliciting dose and symptoms differed when the peanut challenge was repeated, making it impossible to quantitatively estimate the severity of a peanut allergy on the basis of an oral food challenge. However, the reproducibility of a negative or positive test was 100% for both challenge and CD-sens to peanut and Ara h 2.

The correlation between the two CD-sens values was strong. A good correlation does not always mean a high reproducibility, but with a high reproducibility there should be a good correlation. For the peanut challenge we could not demonstrate any statistically significant differences or correlations for doses or symptoms between the two challenges. Thus the low correlation supports that the reproducibility of peanut challenge was poor for eliciting dose and severity score. This is in contrast to CD-sens, where we did not find any significant differences but a significant and strong correlation between the two occasions, supporting that CD-sens results are strongly associated with each other.

We did not find any non-responders to CD-sens, which was surprising since 10-20% of the population has been reported to have non-responding basophils on activation (123), i.e. negative to the positive control (anti-FcεRI). However, in our experience the figure is 5-10% (124). Two children in our study with a positive DBPCFC had basophils with a weak response, <16%, to anti-FcεRI, “low responders”. Thus, in patients with a convincing history of a peanut allergy who are non- or low responders in CD-sens, an oral peanut challenge must be recommended.

Ninety-two percent of the children with a positive DBPCFC had a positive CD-sens to peanut and Ara h 2, and if the low responders were excluded, the concordance with a positive CD-sens was hundred percent.

All children positive in CD-sens to peanut were also positive in CD-sens to Ara h 2. One benefit of using a recombinant allergen like Ara h 2 is that the exact protein content is known. However, pure proteins can sometimes be difficult to handle because of solubility issues and the extremely low concentrations needed. The advantage of using a crude peanut extract is that the same raw material can be used for both CD-sens stimulation and DBPCFC. However, as for other tests using allergen extract, the CD-sens value completely depends on the concentration and the purity of the allergen extract. It is not possible to compare extracts of different allergens, as they are not standardized.

There is a considerable variation in the performance in *flow cytometry assays* (119) and the European Interest Group for evaluation of BAT in clinical use (EuroBAT) is working on a harmonized protocol to make results from different international laboratories comparable (119).

PR-10 proteins

Ara h 8 belongs to the PR-10 protein family and is a homolog of the major allergen in birch pollen (Bet v 1) (105). Sensitization to birch pollen can lead to development of IgE-ab to PR-10 proteins in fruits and vegetables, such as soy (Gly m 4) (156, 157) and hazelnut (Cor a 1) (158-160). Children sensitized to Ara h 8 but not to the storage proteins mostly tolerate peanuts (126) whereas severe reactions have been reported in children with isolated Gly m 4 sensitization (156, 161, 162). The amount of Ara h 8 in peanut is very low and it is also known to have a low stability to heat and gastric degradation (163), which could be a plausible explanation for why children with isolated Ara h 8 sensitization tolerate small amounts of peanut (126). However, a recent study reported that in 1 g roasted peanuts, Ara h 8 represent 8 µg (0.8%) and that

Ara h 8 has some proteolytic stability to gastric and pancreatic degradation, *in vitro* (164).

In paper V, we were able to show that the majority of children with an isolated Ara h 8 sensitization were positive in CD-sens to Ara h 8. This indicates that Ara h 8 has the ability to initiate an allergic inflammation if basophils IgE-sensitized to Ara h 8 become activated by an intact Ara h 8 allergen. The ability of Ara h 8 to cause an allergic inflammation has been shown by others (163). These findings could be a plausible explanation for why the girl with birch pollen allergy and mono sensitization to Ara h 8 in our case report (Paper III) tolerated small amounts (<40 g) of peanut but had a severe reaction after eating a large amount (approximately 300 grams). This observation might indicate that birch-pollen allergic children with cross-sensitization to peanut can tolerate eating some peanuts but need to be careful with larger amounts of peanuts.

We also investigated CD-sens to Gly m 4, in order to compare it with CD-sens to Ara h 8. Previous reports have shown that among birch pollen-allergic individuals, those who are also IgE-sensitized to Gly m 4 report more severe symptoms after drinking soy milk during birch pollen season than those who are sensitized to Ara h 8 who have eaten peanuts (126, 162, 165). The children in our study were selected for having IgE-ab to Ara h 8, but 19/20 also had IgE-ab to Gly m 4. However, only 11 were CD-sens positive to Gly m 4. We did not perform soy challenges but it would be of great interest to investigate if the children with positive CD-sens to Gly m 4 would react at an oral challenge to soy.

6.4 **IgG₄-ANTIBODIES**

In Paper IV we investigated the levels of IgG₄-ab to peanut and peanut components in comparison to DBPCFC. It has been reported that measurement of IgG₄-ab cannot be used to determine if a patient is peanut allergic or not (150, 166, 167) and that IgG₄-ab only seem to be part of a physiological response after prolonged antigen exposure (168). IgG₄-ab concentrations in our study to peanut

and Ara h 2 were low but significantly higher in children with peanut allergy. However, tolerant children had significantly higher ratios of IgG₄-ab/IgE-ab to peanut compared to allergic children. This could indicate a protective role for IgG₄-ab against allergic reactions. IgG₄-ab can bind to mast cells and basophils via the FcγRIIB receptor. Cross-linking between the IgE/FcεRI and IgG₄/FcγRIIB complexes by allergens inhibits the IgE-mediated cell-degranulation (54, 55). IgG₄-ab may also act as blocking antibodies by interfering with the binding between allergen and IgE antibodies (169, 170).

It has previously been reported that IgG₄-ab correlate with IgE-ab levels to peanut and peanut components especially in children avoiding peanuts (171). A similar observation was done in the present study using peanut challenges and the correlation could be due to that both IgE-ab and IgG₄-ab production is dependent on Th2-type cytokine synthesis (51).

All children in this study avoided peanuts thus the levels of IgG₄-ab was not depended on the amount of peanut eaten before the challenge. This could indicate that the IgG₄-ab levels could be associated with peanut allergy and not only to allergen exposure (172).

Hong *et al.* previously published that IgG₄-ab to peanut and its components were not helpful when investigating the severity of peanut allergy since they did not find any differences between allergic and tolerant children (167). In our study there were a significant difference in IgG₄-ab between allergic and tolerant children but the range of the IgG₄-ab levels was too wide to allow the IgG₄-ab to be used as reliable diagnostic markers.

The role of IgG₄-ab has also been investigated, in other studies, in birch-allergic individuals where a high ratio of IgG₄-/IgE-ab to the PR-10 allergen in hazelnut (Cor a 1) or apple (Mal d 1) correlated with tolerance (173). However, there was a high individual variability between patients; hence no recommendation using the antibody ratio in the prognosis of birch-related food allergy was made. In our

study we did not find any differences in the IgG₄/IgE-ab ratio to the PR-10 allergen Ara h 8 between allergic and tolerant children.

6.5 ACCURACY OF PEANUT ALLERGY DIAGNOSIS

A diagnosis of peanut allergy has a great impact on a patient's quality of life. It necessitates a strict diet; the patient must avoid foods containing peanuts because accidental ingestion may result in anaphylactic reactions, which can be fatal (2-4). Prescription of adrenaline auto-injectors is common because no diagnostic method is available to predict the severity of a peanut allergy. All the fear and worry related to the threat of an anaphylactic reaction also impairs quality of life (9-11) and therefore an accurate diagnosis is highly important to avoid serious consequences for the children and their families.

In our first study (study population 1) all 38 children had a suspected peanut allergy and they had been recommended to avoid peanuts before inclusion in the study. Eight of the thirteen children (62%) avoided peanuts before challenge due to peanut IgE-sensitization and eleven children (85%) had been prescribed an adrenaline auto-injector. On the other hand of twenty-five children positive in challenge six of them (24%) had not an auto-injector prescribed. This indicates how difficult it is to diagnose peanut allergy properly. It is also important to avoid screening for peanut allergy without strong clinical suspicion of a clinical reaction to peanut. However, with new diagnostic opportunities like the CRD and CD-sens, the accuracy of peanut diagnoses will improve.

6.6 STRENGTHS AND LIMITATIONS

6.6.1 Study design

The strength of our study design was that all children were sensitized to peanut and had a diet without peanuts. All children were also orally challenged, which is a strength since many other studies rely on results of previous documented reactions. Besides, the challenges were done in the same hospital, Sach's Children and Youth Hospital, where the personnel at the Allergy department

have long experience of performing food challenges. Limitations were that the patient groups are highly selected and not population based, which might have led to a selection bias. The number of patients included was also limited for practical reasons.

6.6.2 Oral peanut challenges

All children in study population 1 underwent DBPCFC. The challenge medium recipe of chocolate balls was scientifically evaluated and contained low levels of low levels of fat (7%). The completeness of challenge testing and the well-characterized challenge medium are two major strengths (128, 174). Another strength is that all challenges were observed and severity scored by the same senior physician. Thus, it is a subjective judgment when objective symptoms occur but also a subjective decision when the challenge is stopped (136). A limitation is the severity scoring, since the score system used did not differentiate OAS (subjective symptoms) from no objective symptoms and furthermore there were no scoring for late reactions. The children in our study were allowed to eat breakfast before the challenges, which may have interfered with the challenge outcome since a large amount of fat eaten before challenge may lead to a milder reaction at challenge.

In paper II we compared the reaction to a DBPCFC with a repeated active challenge to peanut (SBFC). This challenge was blinded for the patients and the parents, but the doctor and the nurses knew that the challenge was active. This knowledge might lead to bias; if the previous reaction had been severe the doctor may have been more careful about provoking objective symptoms at the second active challenge. Another limitation is that children who reacted with severe symptoms at the first challenge were excluded for ethical reasons and did not perform a second active challenge. This might have skewed the results since we do not know how children with severe symptoms would have reacted at a repeated challenge.

We decided not to use DBPCFC when diagnosing peanut allergy in study population 2, which is a limitation. However, we investigated children with suspected cross-reactivity to peanut and did not expect any objective symptoms at challenges. OFC is less time consuming and the method is has also been accepted if the outcome of the food challenge is negative (101, 175). However, if an objective reaction had occurred during OFC, a DBPCFC would have been performed.

6.6.3 **CD-sens**

Major strengths of the CD-sens method are that the basophil threshold sensitivity is measured at eight different concentrations and that this can be done without any risk for the patient. Another strength is that it is possible to use the same peanut raw material in CD-sens as is used in the oral challenges.

Limitations of the CD-sens method are the presence of non-responders and low responders. Other limitations are that the CD-sens method is analyzed manually and the method is only used in a few laboratories in Sweden. Furthermore, since it is a new method, no reference values have been established at the time of these studies. Compared to component resolved diagnostics it is an expensive method. However, the outcome of the test result may be more clinically relevant since the CD-sens assay is a functional assay in contrast to CRD which only shows presence/absence of IgE-ab.

7 ETHICAL CONSIDERATIONS

The studies in this thesis were all approved by the Regional Ethical Review Board at Karolinska Institutet in Stockholm, Sweden and the studies were performed according to good clinical practice based on the Helsinki declaration for clinical investigations. All parents and children gave their informed written consent to participate in the studies before inclusion. They were informed about the study design and the purpose of the study by telephone, and written information was sent home before they began participating in the study. If they agreed to participate an appointment was booked with the doctor and the family was free to ask questions. They were also informed that they could withdraw from the study at any time without any effect on future treatment or care.

8 CONCLUSION

The results of this thesis highlight different diagnostic methods available for a proper peanut diagnosis.

Based on the studies presented, the following conclusion can be drawn:

- CD-sens is a promising diagnostic method with good reproducibility in the diagnosis of peanut allergy and may exclude a peanut allergy.
- An oral peanut challenge can discriminate between a positive and negative challenge outcome, but does not predict the severity of an allergic reaction.
- Component resolved diagnostics is a valuable diagnostic tool in peanut allergy diagnosis since elevated concentration of IgE-ab to the peanut storage proteins (Ara h 1, Ara h 2 and Ara h 3) is associated with peanut allergy.
- Birch-pollen allergic children IgE-sensitized to peanut (only to Ara h 8) have basophils sensitized with Ara h 8 IgE-ab which can be activated by Ara h 8 proteins and initiate allergic inflammation.
- Peanut tolerant children IgE-sensitized to peanuts are characterized by low levels of IgG₄-antibodies but relatively high IgG₄/IgE-antibody ratio to peanut and Ara h 2.

9 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

DBPCFC is considered the gold standard for diagnosis of peanut allergy, but food challenges are time consuming and risky for the patients. Therefore, CD-sens and CRD may contribute with valuable information in peanut allergy diagnosis. CRD can distinguish between genuine peanut allergy and cross-sensitization to the peanut and thus reduce unnecessary anxiety. In individuals IgE-sensitized to peanut, CD-sens may distinguish between presence and absence of tolerance and if CD-sens is negative the probability for an allergic reaction is low.

CD-sens has also proven to be a good biological marker for immunological changes during allergen specific immunotherapy (176). Therefore CD-sens may be used to select patients with high allergen threshold sensitivity to a food allergen, i.e. those who are likely to benefit from oral immunotherapy. In addition, the response to oral immunotherapy may also be monitored with CD-sens (176, 177). Another potential use for CD-sens may be to select and follow patients who would benefit from Omalizumab treatment. The current indication for Omalizumab is severe allergic asthma and chronic urticaria. Dosing of Omalizumab is based on concentration of serum IgE and body weight. However, CD-sens has potential as a way to follow Omalizumab treatment and may be a better way to evaluate treatment response (178-181).

CRD has already been introduced into clinical practice and has improved the diagnostic opportunities in peanut allergy, but more research is needed to investigate the clinical use of CD-sens. Cross-sensitization between different tree nuts is common and could be an interesting research area to explore both with CRD and the CD-sens method. It is also of great interest to continue evaluating the CD-sens method in comparison with oral food challenges in food allergic children.

10 SVENSK SAMMANFATTNING

Bakgrund: Jordnötsallergi kan orsaka svåra, ibland livshotande reaktioner. Att vara allergisk mot ett födoämne innebär att immunförsvaret reagerar på ett i vanliga fall ofarligt ämne och bildar allergiantikroppar (IgE-antikroppar). Individer med benägenhet att bilda allergiantikroppar kan bli sensibiliserade om de kommer i kontakt med ämnet, vilket vid förnyad kontakt kan leda till en allergisk reaktion. I Västeuropa är ca en procent av alla individer jordnötsallergiska, men andelen sensibiliserade är högre och varierar mellan en och elva procent i olika studier vilket kan bero på korsreaktioner mellan olika proteiner som liknar varandra. I jordnöten finns ett protein (Ara h 8) som har liknande proteinstruktur som huvudallergen i björkpollen (Bet v 1). Följden blir att björkpollenallergiska individer kan få allergiantikroppar mot jordnötter (korssensibilisering). Dessa individer kan känna klåda i mun och svalg när de äter jordnötter, men får sällan svåra allergiska reaktioner. En jordnötsallergi diagnostiseras oftast genom anamnes, hudpricktest samt förekomst av IgE-antikroppar i blod mot jordnötsextrakt. Då det är svårt att med säkerhet veta vilka individer med IgE-antikroppar mot jordnöt som har en ”äkta allergi” och därmed riskerar att få en svår allergisk reaktion, behöver diagnosen ofta bekräftas med en jordnötsprovokation. En provokation är tidsödande och kan medföra svåra allergiska symptom. Jordnötsproteinerna, Ara h 1, Ara h 2 och Ara h 3 är stabila lagringsproteiner. IgE-antikroppar mot dessa kan mätas via ett blodprov och anses vara associerade till jordnötsallergi. En annan diagnostisk metod är CD-sens där man fastställer minsta mängd jordnötsprotein som stimulerar basofila celler i provrör. De basofila cellerna medverkar vid uppkomsten av en IgE-förmedlad allergi och via ett blodprov kan man mäta de basofila cellernas känslighet för jordnöt.

Syfte: Det övergripande syftet med avhandlingen var att utvärdera olika diagnostiska metoder hos barn med IgE-antikroppar mot jordnöt som har en misstänkt jordnötsallergi.

Material och Metoder: Trettioåtta barn med misstänkt genomgick tre provokationer blint (jordnötter två gånger och placebo en gång). Vilka symptom barnen fick och mängden jordnötter de kunde äta dokumenterades. Blodprov togs vid de två första provokationerna för CD-sens och IgE-antikroppsanalys. Vi har även undersökt björkpollenallergiska barn med enbart allergiantikroppar mot björk, jordnöt och Ara h 8.

Tjugo barn genomförde en öppen jordnötsprovokation och ett blodprov togs för att undersöka CD-sens mot jordnöt och Ara h 8.

Resultat: Jordnötsallergiska barn hade förhöjda nivåer av IgE-antikroppar mot lagringsproteinerna Ara h 1, Ara h 2 och Ara h 3 samt förhöjda nivåer av CD-sens mot jordnöt och Ara h 2. Alla barn som var negativa i CD-sens tolererade jordnötter. Vi undersökte även reproducerbarheten hos CD-sens metoden och jordnötsprovokationen och fann att CD-sens nivåerna var jämförbara mellan de två mättillfällen medans vid en upprepad jordnötsprovokation reagerade endast tre barn med samma svårighetsgrad och på samma dos. Hos de 20 björkpollenallergiska barnen kunde alla äta jordnötter, men fem barn fick övergående klåda och obehag i munnen som försvann utan behandling. De flesta barn (17/20) reagerade i CD-sens mot Ara h 8 och endast ett barn var positiv i CD-sens mot jordnöt. I avhandlingen beskrivs också en björkpollenallergisk flicka med allergiantikroppar mot Ara h 8 som fick en svår allergisk reaktion efter att ätit 300 g jordnötter hemma. Hon hade tidigare genomfört två stycken jordnötsprovokationer (6.1g) utan att reagera. Flicka äter fortfarande jordnötter (<40 g) men undviker stora mängder.

Slutsats: Utifrån resultatet i denna avhandling verkar CD-sens vara en tillförlitlig diagnostisk metod som skulle kunna utesluta en jordnötsallergi. Förekomst av IgE-antikroppar mot jordnötsproteinerna Ara h 1, Ara h 2 och Ara h 3 bekräftar en jordnötsallergi. Såväl en jordnötsprovokation som CD-sens kan avgöra om en individ är allergisk eller inte. Däremot går det inte med en jordnötsprovokation att förutsäga svårighetsgraden av en allergisk reaktion. Björkpollenallergiska barn har basofila celler som kan aktiveras av Ara h 8 proteiner och starta en allergisk inflammation. Att de flesta inte reagerar kan troligen bero på att andelen Ara h 8 som finns i rostade jordnötter är liten och att den lilla mängden som finns bryts ner i mag-tarmkanalen. Ett stort intag av jordnötter hos en björkpollen allergisk individ som har bildat IgE-antikroppar mot jordnötter på grund av korsensibilisering skulle kunna utlösa en allergisk reaktion. Genom förbättrade diagnostiska metoder kan vi på ett bättre sätt avgöra vilka barn som behöver undvika jordnötter. Många barn med misstänkt jordnötsallergi kan friskskrivas och därigenom få en förbättrad livskvalitet.

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