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IT'S PEANUTS

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ABSTRACT

Allergic diseases are common in the growing population and have been increasing worldwide. Allergic sensitization, i.e. presence of Immunoglobulin E in the blood, is important for development of allergic disease and sensitization to foods often precedes sensitization to inhalant allergens. Peanut allergy is one of the most prevalent food allergies. It is rarely outgrown and is one of the major causes of fatal and near-fatal allergic reactions. However, asymptomatic peanut sensitization is common, but due to the risk of severe reactions, most peanut sensitized individuals have been regarded as peanut allergic from a clinical point of view. As a consequence, this has resulted in decreased quality of life due to fear of severe reactions.

The overall aim of this thesis has been to analyse sensitization patterns to inhalant allergens over time, and to analyse birch pollen- and peanut-IgE antibodies and IgE to peanut allergen components in relation to symptoms of peanut allergy.

The study populations in this thesis emanates from A) 4 089 children from a birth cohort (BAMSE) – with follow up at several time points up to eight years of age, Paper I-III and V, and from B) material from a clinical database, established during 2007-2010 of 237 consecutive children with suspected peanut or tree nut allergy, and attending the outpatient allergy clinic at Sachs' Children's Hospital. Of these children, 98 were included in study V based on sensitization pattern to peanut allergen components.

Paper I describes the dynamic process of sensitization to inhalant allergens. Between four and eight years of age, the proportion of children sensitized to any of the inhalant allergens tested increased from 15% to 25%. At both four and eight years the prevalence of IgE to birch and cat dominated, but sensitization to timothy and dog increased relatively more during this period.

In Paper II we showed that children at school age, sensitized both to birch pollen and peanut are less likely to exhibit high IgE levels to peanut and report symptoms to peanut as compared to children with sensitization to peanut, but not to birch pollen.

In Paper III IgE reactivity to peanut allergen components in 200 eight-year-old children was investigated. Peanut symptoms were reported in 87% of the children with IgE reactivity to any of the storage proteins of the peanut allergen extract Ara h 1, 2 or 3. This is to be compared with 17% of children with IgE reactivity to Ara h 8 (Bet v 1 homologue), but not to Ara h 1, 2 or 3. Furthermore, symptoms were found to be more severe in children with Ara h 1, 2 or 3 IgE reactivity.

Paper IV is a case report from Sachs' Children's Hospital, highlighting that sensitization to Ara h 6, homologous to Ara h 2, even in the absence of this latter protein component may cause severe reactions to peanut. This is likely to occur rarely.

Paper V supports the suggestion that sensitization to Ara h 8 reflects mild OAS or peanut tolerance at oral peanut challenge. However, sensitization to so far unidentified determinants in peanut may in rare cases cause symptoms.

In conclusion, sensitization to inhalant allergens is a dynamic process and birch sensitization dominates at the age of eight. Peanut component Ara h 1-3 sensitization is very often associated with true peanut allergy. Isolated Ara h 8 sensitization seems to indicate peanut tolerance. However, all peanut proteins related to IgE-mediated reactions may not yet have been identified and characterized.

LIST OF PUBLICATIONS

I. **Asarnoj A**, Ostblom E, Kull I, Lilja G, Pershagen G, Hedlin G, van Hage M, Wickman M. Sensitization to inhalant allergens between four and eight years of age is a dynamic process: results from the BAMSE birth cohort. *Clin Exp Allergy*. 2008 Sep;38(9):1507-13

II. **Asarnoj A**, Ostblom E, Ahlstedt S, Hedlin G, Lilja G, van Hage M, Wickman M. Reported symptoms to peanut between 4 and 8 years among children sensitized to peanut and birch pollen - results from the BAMSE birth cohort. *Allergy*. 2010 Feb;65(2):213-9

III. **Asarnoj A**, Movérare R, Ostblom E, Poorafshar M, Lilja G, Hedlin G, van Hage M, Ahlstedt S, Wickman M. IgE to peanut allergen components: relation to peanut symptoms and pollen sensitization in 8-year-olds. *Allergy*. 2010 Sep;65(9):1189-95

IV. **Asarnoj 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*, in press, DOI: 10.1159/000336027

V. **Asarnoj 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. Manuscript resubmitted *J Allergy Clin Immunol*

CONTENTS

LIST OF ABBREVIATIONS

APC	Antigen Presenting Cell
Ara h	Arachis hypogaea (peanut)
ARIA	Allergic Rhinitis and its Impact on Asthma
BAMSE	Children, Allergy, Milieu, Stockholm, Epidemiological Study (Barn, Allergi, Miljö i Stockholm, en Epidemiologisk studie)
Bet v	Betula verrucosa (white birch)
CCD	Cross-reactive Carbohydrate Determinant
CI	Confidence Interval
CRD	Component-Resolved Diagnostics
DBPCFC	Double-blind Placebo-Controlled Challenge
EAACI	European Academy of Allergy and Clinical Immunology
FPIES	Food Protein-Induced Enterocolitis Syndrome
FU	Fluorescent Unit
GINA	Global Initiative for Asthma
Gly m	Glycine max (soy)
HDM	House Dust Mites
ICD	International Classification of Disease
IgE	Immunoglobulin E
IL	Interleukin
ISAAC	International Study of Asthma and Allergy in Children
IUIS	International Union of Immunological Societies
kDa	Kilo Dalton
kU_A/L	Kilo Units (specific allergen IgE) per Litre
LTP	Lipid Transfer Protein
LTP	Lipid Transfer Protein
MHC	Major Histocompatibility Complex
NIAID	National Institutes of Allergy and Infectious Diseases
OAS	Oral Allergy Syndrome
OOFC	Open Oral Food Challenge
OR	Odds Ratio
paO2	Oxygen saturation
Phl p	Phleum pratense
PR	Pathogenesis-related
Pru p	Prunus persica
Q	Questionnaire
RAST	Radio-AllergoSorbent Test (RAST)
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPT	Skin Prick Testing
Th2-cell	type 2 helper T cell
WAO	World Allergy Organization
WHO	World Health Organization

1 INTRODUCTION

1.1 SENSITIZATION, DEGRANULATION AND IMMUNOGLOBULIN E

Allergic disease develops when the immune system reacts to a substance, an allergen which in general is harmless[1]. The allergen is usually a protein in inhaled pollen or animal dander, or a protein from an ingested food item. When the allergen passes the epithelial barrier in the lung, skin or intestine and encounters the cells of the immune system, a process called *sensitization* may take place[2], Figure 1. The allergen is taken up by the antigen presenting cell (APC), which via MHC class II molecule signals to the naïve CD4+ T-cell to develop into a type 2 helper T cell (Th2-cell). The Th2-cell produces cytokines IL-4 and IL-13 and stimulates B-cells to produce allergen-specific Immunoglobulin E (IgE) antibodies. The secreted IgE bind to Fc receptors on mast cells in the connective tissue and on basophil leucocytes in the circulation. The mast cells and basophils are coated with specific IgE – the individual is *sensitized* to that specific allergen. At a following exposure when the same allergen enters the body, the allergen binds to specific IgE on the mast cell and basophil, Figure 2. Cross-linking of bound IgE activates the mast cell or basophil to *degranulate* and release inflammatory mediators, such as histamine, proteases and different cytokines, giving rise to vascular dilatation, smooth muscle contraction, inflammatory cell recruitment and tissue damage. Even in non-allergic individuals the mast cells are coated with IgE, but polyclonal IgE. Thereby, no cross-linking leading to degranulation take place[3].

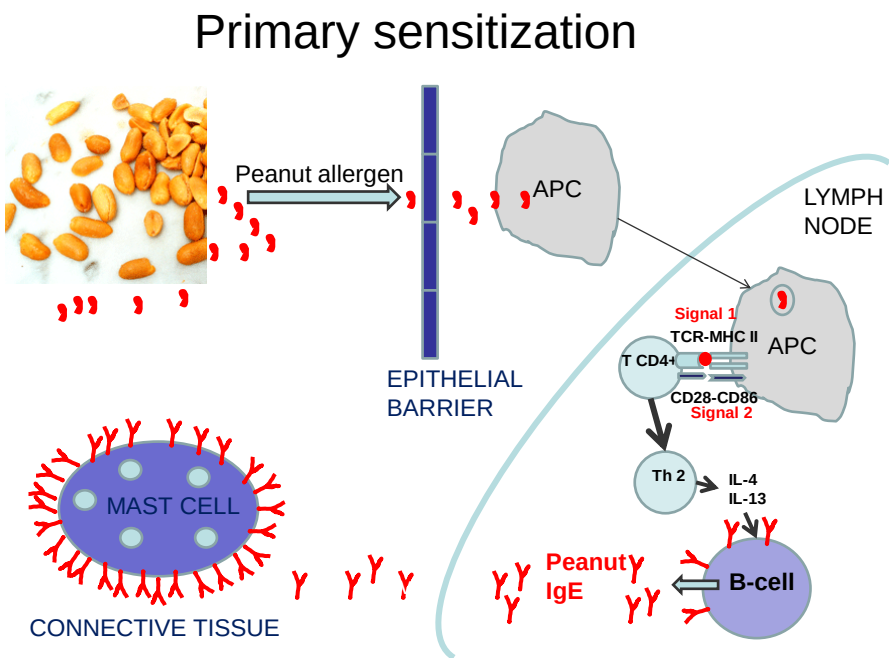

Figure 1. Sensitization exemplified by exposure to peanut allergen. Adapted from Abbas[3] and Rindsjö[2]

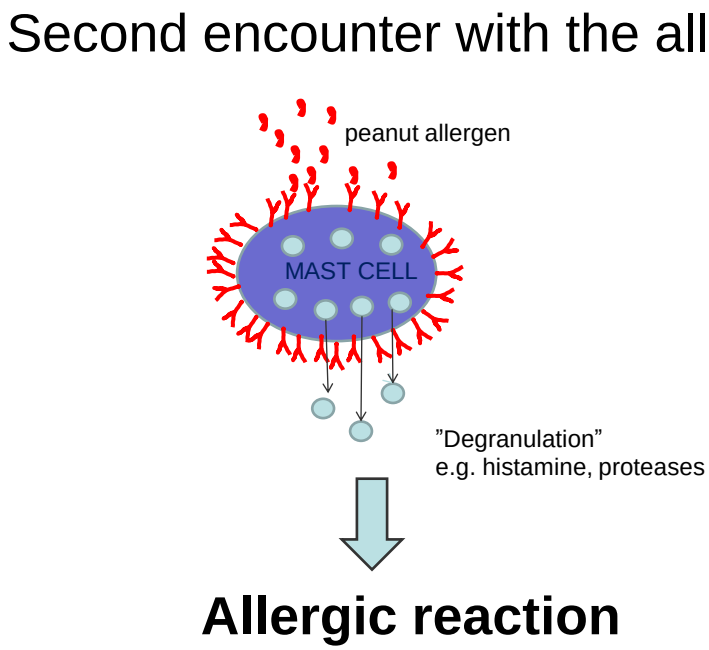

Figure 2. Exposure to peanut allergen in a peanut sensitized individual. Adapted from Abbas[3] and Rindsjö[2]

Why some individuals produce IgE at exposure to a certain allergen, and others do not, is unknown. The important role of IgE in the development of allergic disease is on the other hand well documented[4, 5]. The IgE antibody was discovered in 1967 by two different research groups[6, 7]. The IgE molecule consists of two identical heavy chains and two identical light chains with one variable region on each chain. The four chains are attached together by disulphide bonds into a Y-shaped molecule. The variable regions make antibodies capable of binding many different allergens, including macromolecules and chemical compounds, and each antibody clone is specific for one allergen. However, IgE produced against one allergen may bind to other structurally similar allergens with similar epitopes (= determinants). Such binding is called *cross-reactivity*[3].

1.2 ALLERGY RELATED DISEASES

In allergic individuals, exposure to an allergen against which the individual is sensitized may lead to allergic symptoms. The symptoms can be immediate, within minutes, or rather late and occurring first after 6-24 hours via the mechanisms and mediators described above (1.1). Symptoms may occur from the respiratory tract (asthma, rhinitis, conjunctivitis, laryngeal oedema), from the oral cavity and gastrointestinal tract (OAS, esophagitis, nausea, vomiting, abdominal cramping, diarrhea), from the skin (eczema, flush, urticaria) or from the cardiovascular system (hypotension, bradycardia, syncope, loss of consciousness, death). Anaphylaxis is a term which is used for a multiple organ reaction after exposure to foods, drugs or insects stings[1, 3, 8, 9].

Food allergy, and in particular pollen-related food allergy, is the focus of this thesis.

Clinical characteristics of the different allergic manifestations included in this thesis, are mainly described in the section of methods (3.2.4).

1.2.1 Definitions

1.2.1.1 Asthma

The asthma phenotype is described by the Global Initiative for Asthma (GINA) as "a chronic inflammatory disorder or the airways in which many cells and cellular elements plays a role. The chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment."[10]

1.2.1.2 Rhinitis

The Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines (developed in collaboration with the World Health Organization) defines allergic rhinitis as "symptoms caused by immunologically mediated (most often IgE-dependent) inflammation after the exposure of the nasal mucous membranes to offending allergens. Symptoms of allergic rhinitis include rhinorrhea, nasal obstruction or blockage, nasal itching, sneezing, and postnasal drip that reverse spontaneously or after treatment. Allergic conjunctivitis often accompanies allergic rhinitis."[11]

1.2.1.3 Eczema

Diagnostic criteria of eczema were defined by Hanifin and Rajka in 1980[12]. The definition has been further developed by the United Kingdom Working Party criteria and can be used when a skin condition likely is eczema: Itchy skin condition in the last 12 months plus at least three of, involvement of skin creases, personal history of asthma/rhinitis, generally dry skin, onset before two years of age and flexural dermatitis[13].

1.2.1.4 Anaphylaxis

A position paper for anaphylaxis in children has been prepared by the EAACI Taskforce on Anaphylaxis in Children, defining anaphylaxis as a "severe, life-threatening generalized or systemic hypersensitivity reaction". The clinical criteria are: acute onset, two or more organ systems involved (skin-mucosa/ respiratory tract/ cardiovascular system/ gastrointestional tract) or hypotension after exposure to known allergen[8, 9, 14].

1.2.2 Prevalence and natural course of allergy

Allergic disease in the population worldwide has increased dramatically over the last decades, but seems to level off in the Western world[15-17]. There are large worldwide geographical differences in prevalence of asthma (3%-38%), rhinoconjunctivitis (2%-24%) and eczema (2%-22%)[16].
Allergic symptoms often show a progression throughout childhood from food hypersensitivity and eczema in early childhood to inhalant allergy with asthma and

increasing prevalence of rhinoconjunctivitis in school age. This process is often referred to as "the atopic march"[18, 19]. The natural course of sensitization follows the same route: sensitization to food items in early childhood followed by increasing prevalence of sensitization to inhalant allergens from preschool age and onwards[20, 21]. In Sweden, the prevalence of sensitization to any inhalant or food allergen increases with age from 18%-24% in preschool age[22, 23] to 20%-25% among young schoolchildren[24, 25]. The prevalence of allergic disease in the Swedish paediatric population is about 6%-8% for asthma[24, 26], 5%-17% for rhinoconjunctivitis (increasing with age)[24, 25, 27], 11%-23% for eczema[25, 26] and 2%-8% for doctor diagnosed (14%-24% for self-reported) food allergy[28-30].

1.2.3 Adverse food reactions, food allergy and peanut allergy

According to the 2010 US National Institutes of Allergy and Infectious Diseases (NIAID)-sponsored guidelines, *food allergy* is defined as an "adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food"[31], which means that all adverse reactions from foods are not food allergies. Classification of adverse food reactions are illustrated in figure 3. Except food allergic reactions, food items may cause health effects in different ways: food poisoning with staphylococcus aureus endotoxin is one example of toxic reaction to food[32]. Lactose intolerance is a non-immunologic deficiency of lactase and lead to gastrointestinal symptoms due to undigested lactose[33]. Celiac disease is an immunologic, but non-allergic autoimmune disease, with intolerance to food items containing gluten from mainly wheat[34]. There are also food allergies, which are not IgE mediated, for example contact dermatitis and food protein-induced enterocolitis syndrome (FPIES) with typical symptoms of vomiting, diarrhea and hypotension usually within two hours after ingestion of the food[35]. However, this thesis focuses on IgE-mediated food allergy only and in particular peanut allergy.

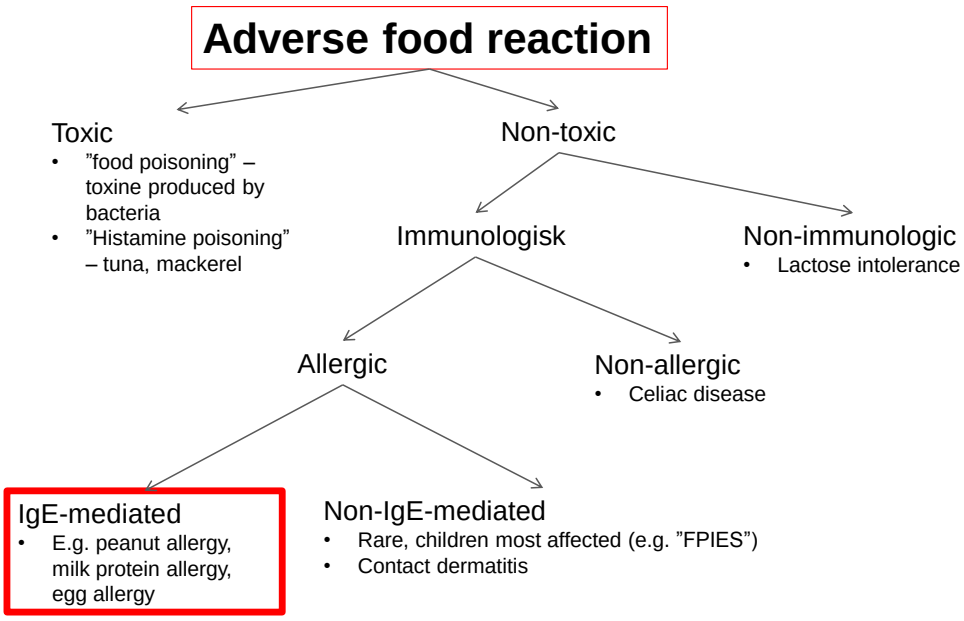

Figure 3. Classification of adverse food reactions (adapted from Burks[35])

Clinical peanut allergy has been reported in 0.2%-1.8% of children and has increased in westernized countries during the last decades[36-40]. The prevalence of peanut sensitization is about 3%-6%[40-42]. Reactions to peanut may be severe[43-45], are usually not outgrown[46-48] and has a negative impact on quality of life[49, 50]. In many countries peanut sensitized children, irrespective if they have experienced allergic symptoms to peanut at ingestion[41, 42, 51], have been advised to avoid peanuts.

1.2.4 IgE cross reactivity between pollens and food items

“Panallergens” is a term used for families of proteins that have similar function and molecular structure and are present in many different types of plants in nature[52]. Cross-reactivity of IgE between members of these families are common, due to their structural similarities. Important plant protein families are the *Pathogenesis-related (PR) proteins* including the Bet v 1 related proteins, the *profilins*, the *prolamin superfamily* with lipid transfer proteins (LTP) and 2S albumin and the *cupin superfamily* with 7/8S and 11S globulins. In order to belong to the same plant protein family the proteins have to be homologous in amino acid sequence or very similar in their biological function and structure[53]. All characterized allergens are listed by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee in a database established in 1984 based on the Linnean system (www.allergen.org).

Oral Allergy Syndrome, or pollen-food allergy syndrome, is since decades a well-known type of food allergy[54]. Typically, a pollen allergic individual experiences symptoms from the oral cavity when ingesting certain raw fruits or vegetables. This phenomenon is experienced by a majority of pollen allergic individuals. Now we know

that symptoms from the oral cavity often is caused by cross-reactivity of pollen IgE to homologous determinants of the symptom eliciting food[53, 55, 56]. However, some individuals exhibit local symptoms from the oral cavity as the first sign of a subsequent systemic reaction[57, 58]. This fact has made the definition of OAS difficult. There is a need for better diagnostic methods to differ between harmless cross-reactions and potentially severe allergy.

1.3 DIAGNOSTIC METHODS FOR FOOD ALLERGY IN GENERAL AND PEANUT ALLERGY IN PARTICULAR

1.3.1 Serum IgE analysis and skin prick testing

During the last decade the field of diagnosing food allergy has developed rapidly. Skin Prick Testing (SPT) was introduced in 1942 and is still widely used[59, 60]. In daily clinical work, SPT is used more or less as a "yes or no answer"; to confirm if there is an IgE response to the allergen or not. However, wheal diameter at SPT correlates with the likelihood of clinical allergy[61-65] but the SPT results are sensible for variation due to circumstances at testing, such as age and skin reactivity[65], but also batch age and skill of the person performing the test . The first commercial serum IgE test, the radio-allergosorbent test (RAST), was introduced in 1972[5, 66]. It utilized solid phase allergens incubated with patient's sera. Bound IgE was detected with a radio-isotopically labeled anti-IgE reagent and radioactivity was measured with a gamma counter. High counts on the gamma counter were proportional to high levels of IgE[5, 66]. The technique developed further into quantitative IgE results by using enzyme labelling and not radioactivity. However, the basic chemistry is the same. Today, there are a couple of companies performing IgE assays, for example Immulite by Siemens Healthcare Diagnostics and ImmunoCAP by Thermo Fisher Scientific (formerly Phadia AB). The ImmunoCAP test is a "sandwich immunoassay"; the allergen of interest is bound to a cellulose capsule and reacts with specific IgE in the sample serum. After washing, reacting IgE molecules are detected by adding enzyme labelled anti-IgE.

1.3.2 Quantitative IgE

In 2001, Sampson et al established threshold specific allergen IgE-levels indicating a 95% likelihood of a clinical reaction to the major food allergens egg, milk, peanut, fish, soy and wheat[67]. Probability curves for likelihood of reaction in relation to specific IgE levels were plotted. The study subjects were paediatric patients attending the Mount Sinai Hospital and were therefore highly selected. The 95% decision point for peanut extract IgE was set to 15 kU_A/L[67]. Since the test is based on crude peanut extract it did not differentiate between sensitization to clinically relevant or harmless allergen components in peanut. Other studies have used similar probability curves to predict allergy[68, 69].

1.3.3 Molecular allergology

1.3.3.1 Molecular allergology in general

The allergen nomenclature is based on the Linnean system (e.g. peanut is named *Arachis hypogaea*) as mentioned. Plant-derived allergens, are named with the three first letters in the genus of the allergen source (e.g. "Ara") followed by the first letter(s) in

the species (e.g. “h”) and an arabic number indicating the chronology of allergen discovery (e.g. “1”).
In 1948 reactions in the mouth and lips were described among patients with rhinitis during spring time[70] and in 1977 an association was shown between birch pollen sensitized patients and positive skin test to for example apple and carrot[71].
In 1991, Ebner et al identified cross-reactivity of the major birch pollen allergen Bet v 1 and with similar protein structures on the apple proteins, explaining a large proportion of OAS in birch-allergic individuals when eating apple. The following decades, the field of molecular allergology advanced and many plant-derived food allergens were molecularly and chemically classified[55]. Two different forms of food allergy were distinguished: one form where the sensitization process to an allergen (stable to gastric digestion) started in the gastrointestinal tract and a second form where prior inhalant allergen sensitization caused cross-reaction to food allergen components, i.e. the food allergy provoking proteins. Another route of sensitization through the skin has also been discussed[72, 73].
The pathogenesis-related (PR) proteins are often involved in cross-reactions between pollen and plant-derived food. There are several types classified in 17 families; birch pollen Bet v 1 and peanut Ara h 8 belong to the PR-10 family. The expression in plants is induced by stress factors such as infection, freezing, wounding or senescence of the plant. They have antimicrobial and toxic effects. They are also present constitutively in many plants and are thought to have other essential functions in plant life except from a defence function[74].
Profilins constitute another panallergen protein superfamily. Like PR proteins, profilins are often causing cross-reactivity between pollen and plant food IgE. Peanut profilin is named Ara h 5. Profilins are actin-binding proteins present in most eukaryotic cells and are important for the cell cytoskeleton. They were first described as allergens in pollens 1991 and later in many fruits and vegetables[75, 76].
Carbohydrate cross-reactive determinants (CCDs) are allergen structures on glycoproteins. CCDs are widely distributed in plants and similar structures are also found on insect venoms[77]. IgE to CCDs are common in allergic individuals but have low biological activity[78].
The most important allergens in many plant-derived food items are the storage proteins which belong to two super-families (cupins and prolamins). They constitute a large proportion of the protein content in many plant foods, in particular in tree nuts, legumes, seeds and cereals[79]. They have a compact 3D structure which make them stable to heat and digestion[80]. Hence, sensitization through the gastrointestinal route is possible.
In 2007 when this thesis project was initiated, allergen components had so far not been used in clinical practice. Although the different routes of sensitization were discussed, the clinical impact of sensitization to different food allergen components had just begun to be understood.

1.3.3.2 Molecular allergology and peanut allergens

In 2007, eight peanut allergens were recognized by the (WHO/IUIS) Nomenclature Subcommittee. Ara h 1 and Ara h 2 were characterized by Burks et al in 1991-1992[81, 82]and Ara h 3 was identified a couple of years later[83, 84]. These three major peanut allergens are all peanut storage proteins of the Prolamin (Ara h 2, 2S albumin) and

Cupin (Ara h 1 7/8S globulin and Ara h 3 11S globulin) super-families. Ara h 4 is nearly identical with Ara h 3[85, 86]. Ara h 5, Ara h 6 and Ara h 7 were identified in 1999[87]. Ara h 5 is a member of the profilins superfamily and Ara h 6 and h 7 (as well as already mentioned Ara h 2) are 2S albumins of the Prolamin super-family. In 2004, Mittag et al characterized Ara h 8 belonging to the PR-10 proteins of which the major birch pollen allergen Bet v 1 is a member[51]. The Ara h 8 protein is 46% identical to birch pollen Bet v 1 in amino acid sequence and therefore Ara h 8 IgE is cross-reactive with Bet v 1[51]. Mittag and co-workers also found out that Ara h 8 was very unstable to gastric digestion and rather unstable to heat. Other researchers have studied the allergenecity of Ara h 1 and Ara h 2 and found them highly stable to heat and digestion and even described enhanced allergenicity after roasting[88-90]. Profilins like Ara h 5 were known as minor allergens of low clinical relevance[56]. However, for example the celery profilin (Api g 4)[91] and the profilin in zucchini[92] have been shown to elicit clinically relevant reactions.

1.3.4 Oral Food Challenges

According to the EAACI position paper on food challenges a food challenge should be performed either to confirm or exclude the diagnosis of food allergy, for scientific reasons, for determination of threshold value of the food or for determining the allergenicity of a food. Food challenges can be performed as an open oral food challenge (OOFC) or as a double-blind placebo controlled food challenge (DBPCFC). In each DBPCFC, neither the patient, nor the doctor or nurse know if the challenge is performed with the food item or with placebo. DBPCFC is the "gold standard" method. An OOFC is often sufficient in children younger than three years, when only objective immediate signs are studied or when there is a high probability of a negative outcome[93]. At peanut DBPCFC, it is important to hide the peanut taste and texture in order to ensure that the challenge is really blind. At the same time, the peanut allergens have to be available, i.e. the blinding must not affect the allergen reactivity. Using vehicles with high fat content is effective for blinding, but reduces the availability of peanut proteins[94]. Recipes for optimizing blinding and allergen availability has been developed[95].

2 AIMS

The overall aim of this thesis was to analyse sensitization patterns over time to inhalant allergens and furthermore to analyse birch pollen- and peanut IgE levels and IgE to peanut components in relation to peanut allergy symptoms.

Paper I: to assess changes in prevalence of IgE and IgE-levels to inhalant allergens between four and eight years of age in a large population-based birth cohort (BAMSE).

Paper II: to investigate reported symptoms of peanut allergy in relation to levels of IgE antibodies to peanut and birch pollen in a cohort of children (BAMSE) who were evaluated at both four and eight years of age.

Paper III: to investigate IgE reactivity to different peanut, birch and grass pollen allergen components and CCD, in relation to symptoms to peanut in children from a Swedish birth cohort (BAMSE) at eight years of age.

Paper IV: in a case report of a 15-year-old boy to illustrate occurrence of peanut Ara h 6 sensitization in the absence of Ara h 2 sensitization and reaction to peanut at exposure.

Paper V: to investigate the risk of systemic reactions at oral challenge with peanuts among children sensitized to peanut component Ara h 8, but not to Ara h 1-3.

3 MATERIALS AND METHODS

3.1 STUDY DESIGN AND STUDY POPULATION

The first three papers of this thesis are based on the birth cohort BAMSE. Paper IV is a case report of a patient at Sachs' Children's Hospital, Södersjukhuset, Stockholm. The fifth paper combines study subjects both from the BAMSE cohort and from Sachs' Children's Hospital.

3.1.1 BAMSE birth cohort

BAMSE is a prospective longitudinal population based birth cohort of 4 089 children[96]. The abbreviation "BAMSE" stands for "Children, Allergy, Milieu, Stockholm, Epidemiological Study" (Barn, Allergi, Miljö i Stockholm, en Epidemiologisk studie). The main original aim of the BAMSE study was to establish risk factors for the development of allergy related diseases in childhood up to the age of four.
Through Child Health Care Centers (barnavårdscentraler), attended by 99.8% of all new born infants the first year of life[97], inclusion of two months old children born between February 11 1994 and November 22 1996 were made. Inclusion was to be closed when a calculated number of 4 000 children was reached. Target population was all children born in certain areas of central and North-Western Stockholm, chosen to be representative of the Stockholm area with suburbs, Figure 4.

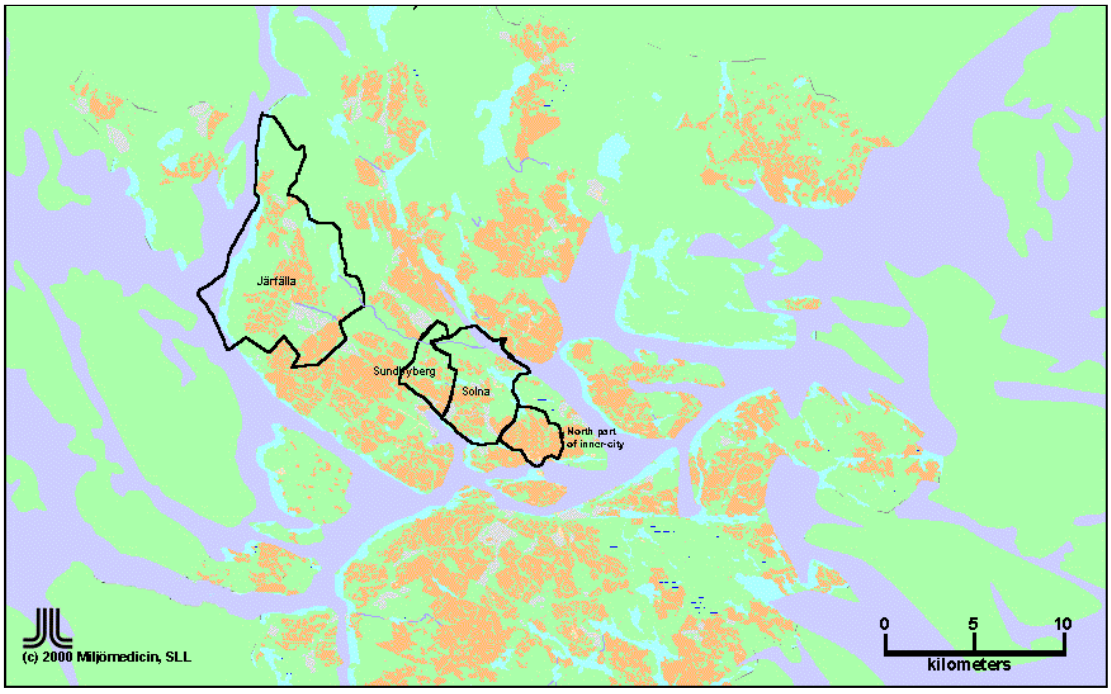

Figure 4. Recruitment areas, BAMSE birth cohort, 1994-1996.

During the recruitment period 7 221 children were born in the specified areas. Of these, 477 (6.6%) could never be reached and another 1 256 (17.4%) were excluded due to the exclusion criteria: plan to move within 1 year, not Swedish speaking, seriously ill child, older sister or brother already included in the study. Of the remaining eligible 5 488 children 4 089 (75%) completed the first questionnaire when the child was about two

months of age and infants enrolled at this time point were defined as the study population, Figure 5.

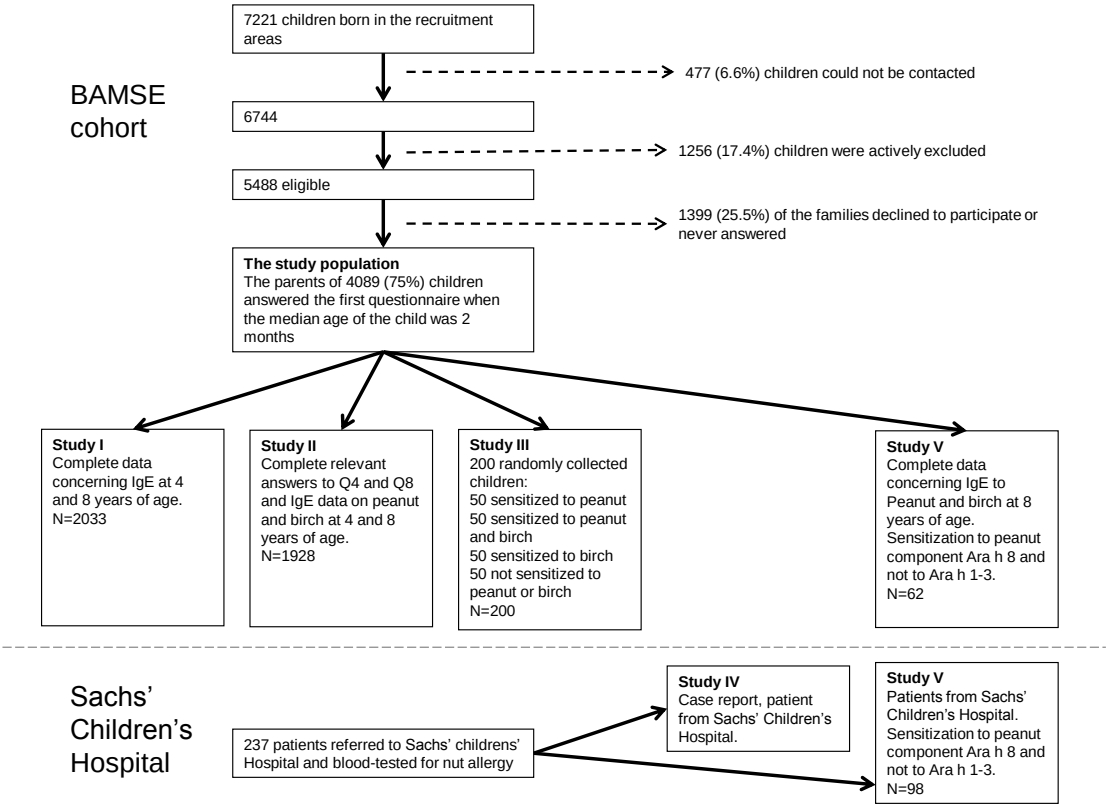

Figure 5.Study design and included study subjects in the five papers of this thesis.

Posted parental questionnaires on parental allergy, lifestyle factors, socio-economic status and environmental factors were completed when the child was two months old (baseline questionnaire). When the child was 1, 2, 4 and 8 years the parents answered questions mainly on the child's symptoms of allergy related diseases. In the cohort of 4 089 children, response rate at each follow up was 96%, 94%, 91% and 84%, respectively. At four and eight years of age all children with completed questionnaires at the current follow up were invited for a clinical examination including blood sampling. From these examinations, sera was available in 2 614 (64%) and 2 461(60%) of the 4 089 children at four and eight years of age, respectively. Later follow ups at 12 and 16 years of age (on-going) were not included in this thesis and will not be discussed further, Figure 6.

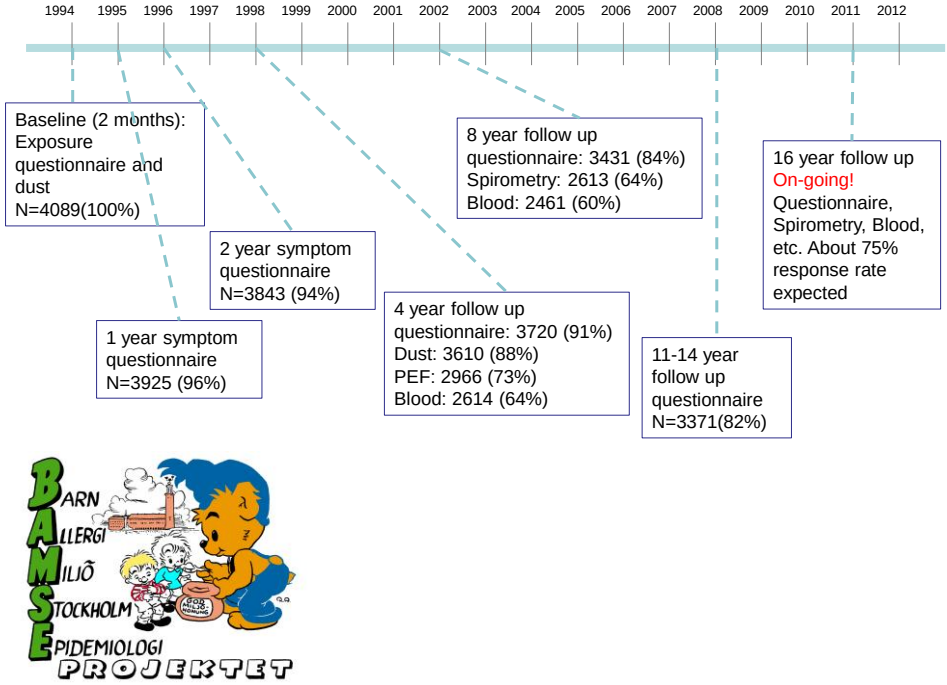

Figure 6. Timeline of the BAMSE study follow-ups 1994-2012.

Non-responders and actively excluded families (N=1 418) were contacted in 1996 with a two pages questionnaire on key exposures (parental smoking, keeping of pets and allergic heredity). Response rates were 58% and 83%, respectively. Parental allergy or keeping of pets did not differ, but parental smoking was significantly higher in the non-responder-excluded group as compared to the families included in the BAMSE study (maternal and paternal smoking 18% maternal and 23% paternal smoking in non-responders vs 9% maternal and 17% paternal smoking in included families)[96].

Study I included only children from which blood was obtained at both four and eight years of age (N=2 033).

Study II included children where data on symptoms to peanut and IgE antibody levels to birch and peanut at both four and eight years of age were available (N=1 928).

Study III had a nested study design: among the 2 480 children from whom blood was drawn at 8 years of age, 200 children representing four different patterns of sensitization to peanut and birch pollen were randomly selected: group A consisted of 50 of 52 children sensitized to peanut, but not to birch pollen; group B consisted of 50 of 141 children sensitized to both peanut and birch pollen; group C consisted of 50 of 237 children sensitized to birch pollen but not to peanut. Finally, group D consisted of 50 of 2012 children with sensitization neither to peanut nor to birch pollen.

Study IV was a case study and did not involve the BAMSE cohort.

Study V included 160 children recruited both from the 8 year follow up of the BAMSE cohort (n=62) as well as patients from Sachs' Children's Hospital (n=98) based on sensitization to peanut allergen components, see 3.2.1.2.

3.1.2 Study subjects recruited from the Sachs' Children's Hospital

The allergy unit at Sachs' Children's Hospital is one of two hospital based allergy units in the Stockholm County with an outpatient clinic managing referrals of allergic children and providing specialized health care for children with moderate to severe allergy related diseases.

Paper IV is a case report of a 15-year-old boy who was referred from the allergy unit of Sachs' Children's Hospital to the Day Ward at the same hospital for an oral peanut challenge.

Paper V: From 2007 until 2010 a clinical database of 237consecutive children, attending the outpatient allergy clinic at Sachs' Children's Hospital with suspected peanut or tree nut allergy, was established. Of the 237 children 192 children had IgE $\geq$0.35 kU_A/L to peanut extract and 98 of these children were included based on their sensitization pattern to peanut allergen components (see 3.2.1.2).

3.2 METHODS

3.2.1 Sensitization

3.2.1.1 Inhalant and food allergen sensitization

The blood samples from clinical examinations at four (N=2 614) and eight (N=2 461) years of age in the BAMSE cohort were screened with Phadiatop® [a mixture of common inhalant allergens: birch, timothy, mugwort, cat, dog, horse, mould (*Cladosporium herbarum*) and house dust mite (*Dermatophagoides pteronyssinus*)] and fx5® [a mixture of common food allergens: cow's milk, hen's egg white, soy bean, peanut, cod fish and wheat] (ImmunoCAP®, former Phadia now Thermo Fisher Scientific, Uppsala, Sweden). Sera with a positive Phadiatop or fx5, defined as IgE levels $\geq$0.35 kU_A/L, were analysed for allergen-specific IgE to the airborne and food allergens listed above. Levels between 0.35 and 100 kU_A/L were registered, and an IgE concentration $\geq$100 kU_A/L was in the statistical evaluation given the value of 101 kU_A/L. Three percent of the analyses for allergen-specific IgE failed due to too scarce amount of blood. The analyses were performed by a certified laboratory (the Clinical Immunology and Allergy Unit, Department of Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm).

Sensitization was defined as presence of IgE to the allergen tested at a level of $\geq$0.35 kU_A/L

3.2.1.2 Peanut allergen component sensitization

In Study III, IV and V, IgE to peanut allergen components in sera was analysed. During the timeframe of the studies the laboratory technique on allergen specific IgE developed with availability of peanut allergen components for IgE testing. This is the reason why different laboratory procedures were used in the different studies.

Microarray: In Paper III IgE to components from peanut (native Ara h 1, h 2, h 3, recombinant Ara h 8), birch pollen (recombinant Bet v 1, Bet v 2), timothy pollen (native Phl p 4, native Phl p 1, 5, 12) and peach (recombinant Pru p 3) as well as

carbohydrate cross-reactive determinant (CCD) were measured using an experimental in-house microarray developed and run by Phadia AB, Uppsala, Sweden. The purified allergen components were spotted on nitrocellulose membranes on microscope glasses. 30 µl sample serum was added and incubated. After washing, bound IgE antibodies were detected with fluorescent anti-IgE and fluorescence intensity was measured at wavelength 635 nm[98]. Individual cut-off levels were established for each allergen, based on background fluorescence from negative samples, and ranges between 400 and 600 FU.

ImmunoCAP®: In paper IV and V the common ImmunoCAP test (description, see 1.3.1) was used to analyse IgE to allergens of peanut protein components, birch pollen, Bet v 1 and CCD. At the time of inclusion of patients in study V, the peanut allergen components Ara h 1, h 2, h 3, h 8 and h 9 were commercially available (recombinant extracts). At the time for challenge of the patients in paper IV and V the 2 S albumin Ara h 6 (sequence Acc. No. Q647G9) was available in a limited amount at former Phadia AB, Uppsala. Recombinant Ara h 6 was produced in *Escherichia coli* as a hexahistidine-tagged recombinant protein using a synthetic gene construct and purified by immobilized metal ion affinity chromatography followed by ion exchange chromatography, as described[99]. Experimental ImmunoCAP Ara h 6 tests were prepared[100].

Of the 2 480 children from the BAMSE cohort with blood samples at eight years of age, 195 were sensitized to whole peanut extract i.e. peanut IgE≥0.35 kU/L. All these children were further analysed with ImmunoCAP in November 2009 for IgE to available peanut allergen components and 62 of these were sensitized to Ara h 8 but not to Ara h 1, h 2 or h 3 and included (Ara h 8 IgE≥0.35 kU/L and Ara h 1-3 IgE<0.35kU/L) in study V.

In blood samples from the 237 children from Sachs' Children's Hospital peanut components Ara h 1-3 and Ara h 8 were analysed (ImmunoCAP) in clinical routine. 98 patients were sensitized to Ara h 8, but not to Ara h 1, h 2 or h 3 and were therefore included in study V.

3.2.2 Study subject characteristics

3.2.2.1 Questionnaires

Paper I-III: In the BAMSE study we used answers from parental questionnaires on parental allergy, lifestyle and environmental factors when the child was two months old (baseline questionnaire). Data from the questionnaires at one, four and eight years were also used, in which the parents answered questions on symptoms of the child's allergic diseases, medications and breast-feeding. In the questionnaires for four and eight years of age, specific symptoms on reactions to peanut and birch pollen were asked for as well as active avoidance of peanut.

Paper IV-V: Parents of included children from Sachs' Children's Hospital in study V received questions by posted mail concerning current peanut consumption similar to the questions used in the 8 year of age BAMSE questionnaire. Those who did not answer

via returning posted mail or email were asked the same questions via telephone calls by physician or nurse.

3.2.2.2 Medical records (Paper IV-V)

In Paper IV, the case report, details of clinical history and laboratory details were retrieved from the patient's medical records at Sachs' Children's Hospital.

In Study V, a history of systemic reaction at peanut exposure was retrieved from medical records when present and was also used for evaluation of reaction severity. Furthermore, data on doctor's diagnosis of asthma, allergic rhinitis/rhinoconjunctivitis, eczema and food allergy, other than allergy to peanut, were also retrieved from the same medical records. The International Classification of Disease, tenth revision (ICD-10) was used.

3.2.2.3 Clinical investigations (Paper I-V)

Paper I-III and V: At four and eight years, all families with completed questionnaires in the BAMSE cohort were invited to a clinical examination including pulmonary function tests and blood sampling. At four and eight years, 2 614 (70%) and 2 461 (72%), respectively, of the invited families participated in a clinical examination (including blood sampling). The examination was performed at the Department of Occupational and Environmental Health, Stockholm County Council, by paediatric nurses.

Paper IV-V: All children who underwent oral peanut challenge at Sachs' Children's Hospital were interviewed and examined by a physician before challenge. Heart- and lung-auscultation and inspection of oral cavity were performed and blood pressure was measured before challenge and at challenge if symptoms occurred.

3.2.3 Oral peanut challenges (Paper IV-V)

Paper IV: The patient in the case report took part in a pre-study in order to evaluate a three dosing steps procedure at open oral challenge (0.1 + 1 + 10 g of roasted peanuts at 20 minutes interval). This was done in patients where no reactions were expected.

Paper V: Oral challenge was performed in children without current peanut consumption.
Inclusion criteria for oral challenge: Children with current sensitization to Ara h 8 but not to Ara h 1, h 2 or h 3 and with no previous exposure to peanuts, avoidance of peanuts for different reasons or anaphylaxis grade I or milder after previous exposure to peanuts.
Exclusion criteria for oral challenge: Children with anaphylaxis grade II-III after previous exposure to peanuts[8]. Children with on-going allergic reaction to other food and inhalant allergens were also excluded.
Peanut tolerance: Children with reported peanut consumption at least at one occasion in the last 12 months without symptoms or discomfort.

3.2.3.1 OOFC (Open Oral Food Challenge), paper V

An open oral food challenge (OOFC) was performed in four stages: 100 mg, 1 g, 5 g and an additional 5 g of pure roasted peanut with 20-minutes intervals, total amount 11.1 grams of peanut equivalent to 13-15 peanuts, followed by one hour observation period after the final dose. Before the challenge, an intravenous catheter was administered on all children, under local anaesthesia (EMLA®) when wanted.

3.2.3.2 DBPCFC (Double Blind Placebo Controlled Food Challenge)

In cases of a non-systemic or systemic reaction up to anaphylaxis grade I at OOFC or a previous documented systemic reaction up to anaphylaxis grade I at peanut exposure, a DBPCFC was performed[95, 101]. Double-blind, placebo-controlled food challenge was performed by an experienced nurse using a challenge medium (chocolate brownie like cookie) containing 11% peanut and 7% fat in increasing doses every 30 min in five steps from 1 mg to 5 g peanut[95], followed by a 2-hour observation period after the final dose. The code for un-blinding was kept sealed for the study object, nurse and doctor until both challenges (active and placebo) were completed.

3.2.4 Definition of outcomes

3.2.4.1 Paper I

The first study had a descriptive approach and main outcomes were sensitization to different allergens at four and/or eight years of age. Definition of sensitization, see paragraph 3.2.1.1 above.

When the study group of BAMSE children in this thesis was compared with the children of the BAMSE study base (the original cohort) information on asthma, rhinitis, eczema, exclusive breastfeeding, parental smoking, cat ownership and parental allergy was used. The comparison was done in order to determinate if there were differences in background factors between the study group and the entire cohort. Definitions were based on questionnaire answers at 2 months (Q0), one year (Q1), four years (Q4) and eight years (Q8) as follows:
Asthma: Q4 and Q8, respectively; Parental report of at least 4 episodes of wheeze in the last 12 months or at least one episode of wheeze during the same period, combined with prescription of inhaled steroids[102].
Rhinitis: Q4 and Q8, respectively; Fulfilling the ISAAC definition of rhinitis – parental report of prolonged rhinitis (sneezing or a runny or blocked nose) without common cold the last 12 months prior to questionnaire[16].
Eczema: Q4 and Q8, respectively; Parental report of dry skin in combination with itchy rash for two weeks or more AND typical localization (face or arms/legs flexures or wrists/ankles or neck) in the last 12 months prior questionnaire AND/OR doctor's diagnose of eczema since previous questionnaire[102].
Exclusive breastfeeding: Exclusively breast fed 4 months or more (Q1)[103].
Parental Smoking: Any of the parents smoked at least one cigarette per day at the time of Q0[104].
Cat ownership: Had a cat at home at the time of Q0[104].

Parental allergy: Mother AND/OR father with doctor's diagnose of asthma and asthma medication AND/OR doctor's diagnose of hay fever in combination with furred pets- and/or pollen-allergy at the time of questionnaire Q0[105].

3.2.4.2 Paper II

Main outcomes were symptoms to peanut at four and eight years of age, respectively. Secondary outcomes were symptoms to birch at four and eight years of age, respectively.

Questionnaire data, at four and eight years of age and obtained prior to blood-sampling were used. Symptoms to peanut and birch were defined as a positive answer to any of the following questions in the four or eight year of age questionnaires:
Symptoms to peanut at four years: 'After two years of age, has your child at any occasion experienced any problems from eating peanuts such as vomiting, diarrhea, eczema, urticaria/itching rash, swollen lips/eyes, itchy, blocked or runny nose or asthma?'
Symptoms to peanut at eight years: 'Is your child allergic to peanuts'. If yes, symptom options were 'nose/eye symptoms', 'mouth-itching', 'breathing difficulties', 'vomiting/diarrhea', 'eczema', 'urticaria' or 'excluded because of early symptoms'. Peanut had to be indicated for at least one of these symptoms.
Symptoms to birch at four years: 'Has your child at any occasion after two years of age experienced problems with wheeze, cough, itching skin rash, sneezing, runny or blocked nose or red itchy eyes when trees are leafing (month of May)?'
Symptoms to birch at eight years: 'Has your child experienced wheeze or disturbing cough, itching eczema or sneezing, runny or blocked nose or red itchy eyes related to birch pollen?'

As in paper I, background factors and current allergy related disease (sex, parental allergy, asthma, eczema, rhinitis, inhalant allergen IgE levels) in the study population (N=1 928) were compared with the same data of children in the original cohort at two months and eight years of age. The same definitions were used as in paper I (see 3.2.4.1).

3.2.4.3 Paper III

Main outcome was reported symptoms to peanuts at eight years of age as a dichotomous outcome. Secondary outcome was type of symptom(s) to peanut or peanut tolerance.

Peanut-symptomatic children
Positive answer was required to the following question in the eight-year-questionnaire regarding the latest 12-month period: 'Is your child allergic to any food item?' Symptom options were 'nose/eyes symptoms', 'mouth-itching', 'breathing problems', 'vomiting/diarrhea', 'eczema', 'urticaria'. Reactions to peanut had to be indicated on at least one of these symptoms or reported as 'excluded from the diet during the last 12 months because of previous symptoms'. Symptoms from nose/eyes or oral cavity were considered as mild symptoms, whereas wheeze or dyspnoea were considered as severe

symptoms[14]. Gastrointestinal symptoms, eczema and urticaria were not possible to classify according to severity from questionnaires.

Peanut-tolerant children
Children with none of the symptoms mentioned above in relation to peanut consumption and reported by the parents during the latest 12 months prior to Q8.

As in paper I, background factors and current allergy related disease (sex, parental allergy, asthma, eczema, rhinitis, inhalant allergen IgE levels) in the study population (N=200) were compared with the same factors of all children participating in the eight years of age follow up. The same definitions were used as in paper I.

3.2.4.4 Paper IV

The described anaphylaxis was graded according to a modified definition of the EAACI position paper on anaphylaxis in childhood[8, 14]. Two or more organ systems needed to be involved in the reaction. Severity score (grade I-III) was based on the organ system most affected.

3.2.4.5 Paper V

Main outcome in the peanut challenge study was outcome at oral peanut challenge.

Peanut tolerance: Children with reported peanut consumption at least at one occasion in the last 12 months without symptoms or discomfort (not challenged). This could be confirmed of all children recruited from Sachs' Children's Hospital and thus they were regarded as tolerant (n=31). The 47 peanut tolerant children included from the BAMSE cohort (definition see outcomes, Paper III) were not contacted further for logistic reasons, they were considered tolerant at the time of inclusion in paper V (eight years of age).

Outcomes of the oral food challenge:
Negative outcome:

- No objective symptoms during 60 minutes after last dose of peanut at oral provocation and no parental report of late symptoms 24 h after challenge.
- Oral allergy syndrome (OAS), i.e. local symptoms from the oral cavity - itching and tingling of the lips, mouth, and throat but without skin symptoms, breathing difficulties or tissue swelling[60].

Positive outcome:

- Systemic reaction with manifestations from the cerebrovascular system, gastro-intestinal tract, lower respiratory tract or skin. For classification of a reaction as anaphylaxis, symptoms were required to be present from the cerebrovascular system or at least two of the following organ systems: gastro-intestinal tract, upper or lower respiratory tract or skin. Severity score was based on the organ system most affected[8, 14].

3.3 STATISTICAL ANALYSES

In Paper I-III the included children were compared to the BAMSE study base (the entire cohort) with respect to background factors at inclusion (sex, parental allergy, parental smoking and cat ownership) and at follow-up at four and eight years of age (asthma, rhinitis, eczema, IgE-levels to inhalant and food allergens). Dichotomous variables were tabulated on proportions and 95% Confidence Intervals (95% CI) were calculated. Intervals that did not overlap were considered as statistically different. Continuous variables (IgE-levels) had a skewed log distribution and were subject to a logarithmic transformation before group comparisons with Student's t-test.

Paper I-III and V used the following statistical measures and analyses: Prevalence was expressed in total numbers and percentages. 95% CI were calculated when appropriate and intervals that did not overlap were considered as statistically different. Chi-square test was used for statistical comparisons of dichotomous variables. Fisher's exact test was used if one comparison group consisted of 5 observations or less. Allergen-specific IgE levels were log normally distributed and subject to a logarithmic transformation before analysis. Student's t-test was used (on the logarithmic scale) for analysis of the continuous IgE-variables. Mean IgE levels were presented as geometric mean (and 95% CI) in Paper I-III and as median (and range) values in Paper V.

Statistical analyses specific for the different papers:
In Paper I, Multiple logistic regression models were used for calculations of odds ratios (ORs) and 95% CI between specific sensitizing allergen at 4 years in relation to sensitization at 8 years[106]. Adjustments in the multivariate analyses were made for parental allergy and sex, which affected the risk of sensitization to the outcome allergens tested with 10% or more. Because allergic children are more likely to be sensitized to multiple allergens, adjustments were made for each inhalant and food allergen tested at 4 years.

In Paper II, the relationship between symptoms to peanut and IgE antibody levels was estimated using a logistic regression model. The odds ratios (ORs) were estimated using logistic regression models and 95% CI were generated. Fitted predicted probability curves according to the levels of the specific IgE to peanut were plotted using the results from the logistic regression. All tests were according to Wald[106]. Logistic regression was also used to investigate peanut symptoms in relation to sensitization to birch and timothy. Several models for identifying confounders were run. Among others, variables of heredity, sex and exposure to tobacco smoke were tested, but since they did not confound the results by 10% or more, they were not included in the analyses.

In Paper III Spearman's rank correlation test was used to establish the strength of relationship between specific IgE antibody responses in the microarray.

Paper IV did not contain any statistical analyses.

Paper V did not contain any statistical method specific for this study (see above, second paragraph).

In all studies, p-values <0.05 were considered significant. All statistical analyses were performed with STATA Statistical Software (release 9 and 11; StataCorp, College Station, Texas, USA).

3.4 ETHICAL PERMISSIONS

Ethical permissions for all studies were received from the regional ethics review board at Karolinska Institutet, Stockholm, Sweden. Register numbers for permissions were:
Study I: 93-189, 98-175, 02-420
Study II: 93-189, 98-175, 02-420
Study III: 93-189, 98-175, 02-420
Study IV: 2012/99-32
Study V: 93-189, 98-175, 02-420, 2010/1331-31/3, 2012/99-32

Informed consents were obtained from the parents, and in some cases also the child, in all families participating in the studies.

4 RESULTS

In Paper I-III, background data of children selected in the three studies were compared to the same data of children in the BAMSE study base in order to investigate if any selection bias existed in the study groups. Children in the study groups did not differ significantly in sex, parental allergy or prevalence of asthma, eczema, rhinitis or in levels of specific IgE (assessed using Phadiatop and fx5) at four or eight years of age compared to remaining children in corresponding age group or in important background factors of the study base in any of the studies (data not shown).

4.1 SENSITIZATION TO INHALANT ALLERGENS BETWEEN FOUR AND EIGHT YEARS OF AGE (PAPER I)

At four years 309 (15%) and eight years 510 (25%) of the 2 033 children were sensitized to at least one inhalant allergen ($p<0.001$). The prevalence of positive (IgE ≥0.35 kU/L) tests and the IgE concentrations, presented as geometric mean, of the selected inhalant allergens in relation to age are presented in Figure 7. The prevalence increased significantly with age for all allergens. Both at four and eight years, birch pollen followed by pollen of timothy, cat and dog dander were the most common sensitizing allergens.

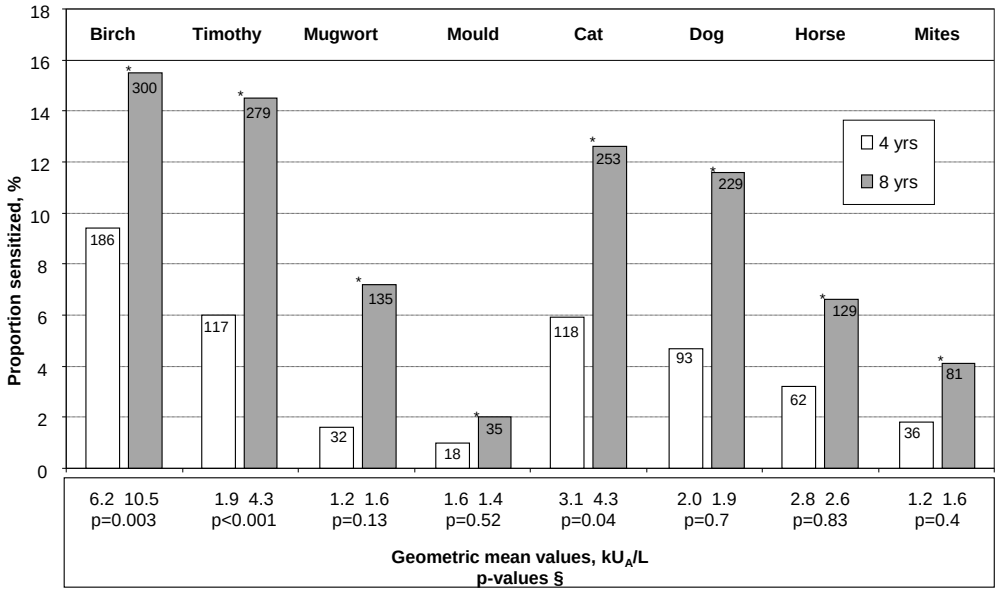

Figure 7. Prevalence of sensitization (IgE≥0,35 kU$_A$/L) and geometric mean values in kU$_A$/L to specific airborne allergens at four and eight years of age in 2 033 identical children.
§ p-values for difference in group IgE levels at eight years as compared to four years of age.
* p<0.01 difference in prevalence at eight years as compared to at four years of age. *Asarnoj et al, Clin Exp Allergy. 2008 Sep;38(9):1507-13*

Sensitization at four years of age to common inhalant allergens was persistent in most children at eight years of age (Table 1). This was true for 97% of the children sensitized to pollen of birch, 86% to timothy, 97% to mugwort, 94% to dander of cat, 91% to dog and 94% to horse at four years of age. Among these children all the allergen-specific IgE-levels increased significantly ($p<0.01$) except for IgE levels of mould and mite allergen. Among children with remittent sensitization, i.e. sensitization at four, but not at eight years, only 38% of the children were mono-sensitized and as many as 20% of the children were sensitized to 4 inhalant allergens or more. The allergen-specific levels of all 48 children who remitted ranged from 0.35 to 3.92 kU_A/L, geometric mean 0.75 kU_A/L.

Table 1. IgE antibody levels (geometric mean) to inhalant allergens among children sensitized at four or eight years only or sensitized both at four and eight years.† *Asarnoj et al, Clin Exp Allergy. 2008 Sep;38(9):1507-13*

	At 4 but not at 8 years in 48 children			Both at 4 and 8 years in 279 children				At 8 but not at 4 years in 426 children		
			IgE			IgE at 4 ϕ	IgE at 8 §			IgE
Allergen	N	%	g.mean (kU_A/L)	N	%	g.mean (kU_A/L)	g.mean (kU_A/L)	N	%	g.mean (kU_A/L)
Birch	5	0.3	0.9	181	8.9	6.5***	21.3***	119	5.9	3.4
Timothy	16	0.8	0.8	101	5.0	2.2***	10.0***	178	8.8	2.6
Mugwort	1	0.1	0.5	31	1.5	1.2***	3.3***	104	5.1	1.3
Cat	7	0.3	0.7	111	5.5	3.3***	8.1***	142	7.0	2.6
Dog	8	0.4	0.6	85	4.2	2.2***	5.0***	144	7.1	1.1
Horse	4	0.2	0.7	58	3	2.9**	5.1***	71	3.5	1.5
Mites	11	0.5	0.9	25	1.2	1.4 NS	1.9 NS	56	2.8	1.4
Mould	6	0.3	0.7	12	0.6	2.2 NS	2.2 NS	23	1.1	1.0

† Internal missing: 0.9%-2.2% (of 2033 children) for each allergen tested.
§ For all allergens, except mould and mites, the IgE antibody levels were significantly higher among those with persistent sensitization compared to those with late onset sensitization.
ϕ For all allergens, except mould and mites, the IgE antibody levels increased significantly between 4 and 8 years among those with persistent sensitization.
**p<0.01, *** p<0.001, NS= Not significant.

Among eight-year-old children we analysed the association between specific IgE levels (kU_A/L) to the pollens and furred animals and the number of allergens (pollen and/or animal species) that the child was sensitized to. With increasing number of sensitizing

pollens, the IgE levels to each of birch and timothy pollen increased. This pattern was also seen for sensitization to furred pets.

We further analysed sensitization to inhalant and food allergens at four years of age and the odd ration for persistent or onset of sensitization to the 4 most common inhalant allergens (birch, timothy, cat and dog) at eight years of age. A multiple logistic regression model adjusting for identified confounders such as sex and parental allergy, but also for all allergens tested, was used. The odds ratios decreased after adjustment for the other allergens which was most pronounced for IgE to mould spores, mugwort pollen and horse dander, but comparably less for birch pollen and fx5. Sensitization to any pollen among the four-year-olds increased the odds for sensitization to birch and timothy at eight years. Furthermore, sensitization to birch pollen increased the odds ratios significantly for sensitization to both cat and dog at eight years.

4.2 REPORTED SYMPTOMS TO PEANUT BETWEEN FOUR AND EIGHT YEARS AMONG CHILDREN SENSITIZED TO PEANUT AND BIRCH POLLEN (PAPER II)

At four years, 106 (5.5%) of the children were sensitized to peanut and 181 (9.4%) were sensitized to birch. At eight years the corresponding proportions were 142 (7.4%) and 293 (15.2%), Figure 8. From four and eight years the proportion of children sensitized to peanut, but not birch (subgroup 1), remained essentially unchanged, 2.2% and 1.9% at the two respective ages, whereas the proportion of children sensitized to both peanut and birch pollen (subgroup 2) seemed to increase over the same period (3.3% and 5.4%, respectively, p=0.002), Figure 8.

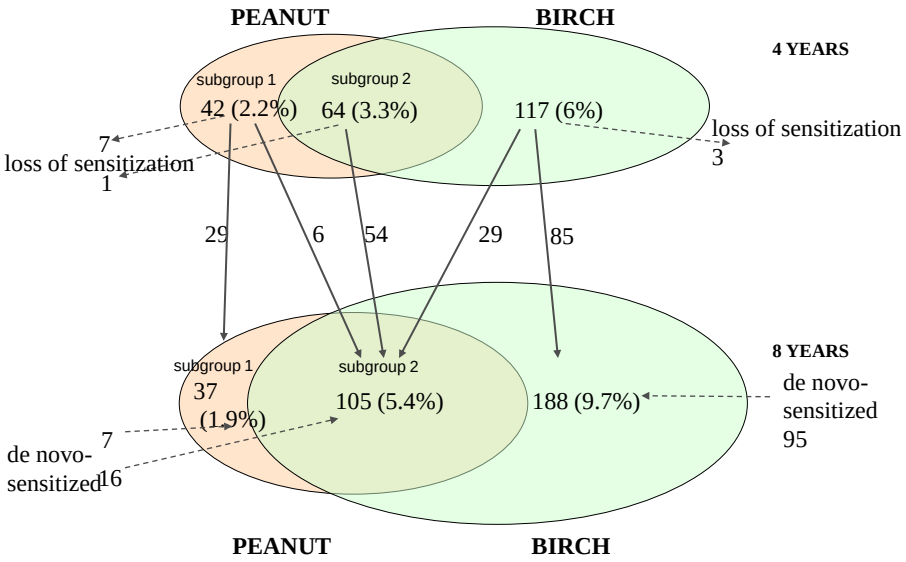

Figure 8. Changes in sensitization to peanut and birch pollen between four and eight years of age among 1 928 children living in Stockholm. *Asarnoj et al, Allergy 2010 Feb; 65(2): 213–9.*

At four years 2.9% (n=56) of the 1 928 children reported symptoms to peanut and at eight years the corresponding proportion was 5.7% (n=109).The prevalence of sensitization to peanut in combination with reported symptoms to peanut was 1.8% (n=34) at four years and 3.9% (n=76) at eight years of age.
At four years of age the proportion of children reporting symptoms from peanut did not differ among peanut sensitized children with or without concomitant sensitization to birch pollen (subgroup 2 vs subgroup 1); 34% vs 29% (p=0.53). However, at eight years of age 76% of the children who were sensitized to peanut but not to birch pollen (subgroup 1) reported symptoms to peanut, whereas among children sensitized both to peanut and birch pollen (subgroup 2) only 46% reported such symptoms, p=0.002. All types of peanut-related symptoms at eight years of age (see definition of symptoms 3.2.4.2), except eczema, were reported more frequently among children in subgroup 1 than in subgroup 2, Figure 9. Significant differences were seen for breathing difficulties; 27% vs 10%, p=0.008 and urticaria; 16% vs 6%, p=0.048). However, 19 peanut and birch pollen sensitized children reported systemic reactions.

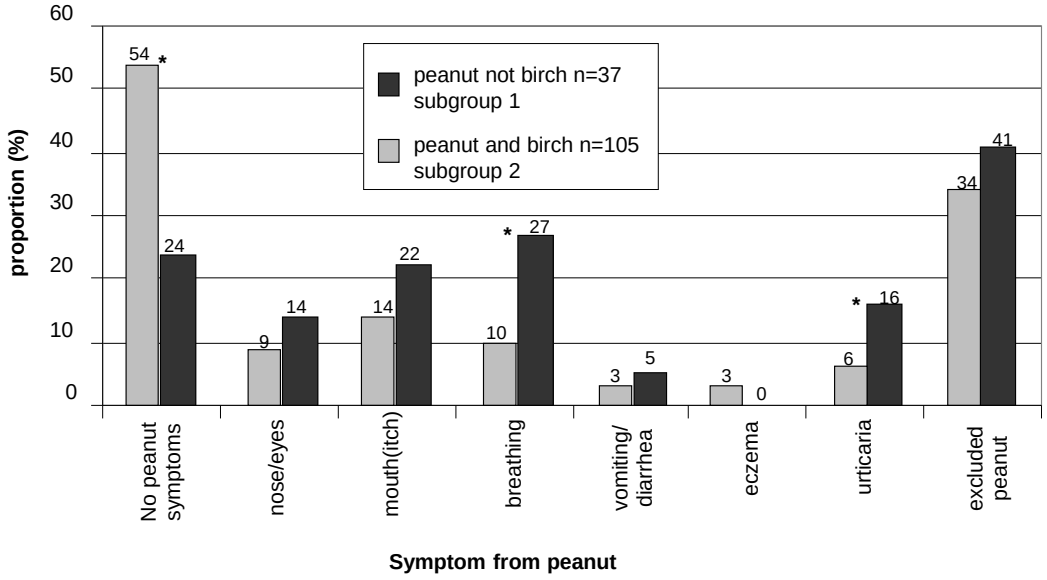

Figure 9. Proportion of different symptoms from peanut among 142 eight-year-olds sensitized to peanut but not to birch or peanut and birch. Figures above bars represent the absolute numbers of children. *p<0.05.

It is noteworthy that children de novo-sensitized to peanut between four and eight years (n=52) were mostly asymptomatic (85%) to peanut and concomitantly sensitized to birch pollen (87%).

At four years of age, although the difference was not statistically significant, the mean IgE level to peanut was higher among children sensitized to peanut and not birch pollen

(subgroup 1) than among children sensitized to both peanut and birch pollen (subgroup 2), 8.2 kUA/L (95% CI 4.5-14.9) and 4.5 kUA/L (2.9-6.9), respectively, p=0.093. However, at eight years this difference in IgE antibody concentrations was significant: 12.0 kUA/L (6.1-23.9) and 4.3 kUA/L (3.1-6.0), respectively, p=0.003).

Sensitization to timothy did not independently influence on peanut symptoms (data not shown).

When the children were four years of age, 13 of them had not yet been exposed to peanuts. Exclusion of these 13 children from the analysis did not change the results.

4.3 IGE TO PEANUT ALLERGEN COMPONENTS: RELATION TO PEANUT SYMPTOMS AND POLLEN SENSITIZATION IN 8-YEAR-OLDS (PAPER III)

4.3.1.1 Component sensitization within the four groups

The four groups (A- sensitized to peanut, B - sensitized to birch and peanut, C - sensitized to birch and D - not sensitized to peanut or birch) differed substantially regarding the IgE reactivity to Ara h 1, 2, 3 and 8, CCD and profilin (Bet v 2/Phl p 12) with the use of microarray technique, Figure 10. IgE reactivity to Ara h 1, 2 and 3 was most common in group A. No individual had IgE reactivity to Ara h 1 or Ara h 3 without an associated Ara h 2 sensitization. No child in group A had IgE reactivity to Ara h 8. IgE reactivity to the peanut storage proteins (Ara h 1, 2 and 3) was present only in children sensitized to peanut (group A and B), whereas 11 children in group C (birch pollen sensitized only) had IgE reactivity to Ara h 8. IgE antibodies to Ara h 8 were common in children sensitized to birch pollen (group B and C; 38% and 22%, respectively). The prevalence of IgE reactivity to CCD was low in all groups, ranging from 0 to 18%, and was most prevalent in group B. Similarly, none of the children in group A and B had IgE antibodies to LTP, i.e. Pru p 3 (peach LTP used as a crude marker of Ara h 9).

When the IgE level to peanut extract was investigated in relation to individual IgE reactivity to the peanut allergen components, children with reactivity to Ara h 1, 2 or 3, but not to Ara h 8, had significantly higher IgE antibody level (geometric mean, 95% CI) to peanut extract (18.9 kUA/L , 11.5-31.0 kUA/L, n=46) than children with reactivity to Ara h 8 only (1.0 kUA/L, 0.60-1.7 kUA/L, n=23).

Of the 100 peanut-sensitized children (group A and B), 25 were to our surprise IgE negative to all tested peanut components in the microarray including CCD and profilin (Fig. 10). In 17 of these 25 children, the peanut-specific IgE levels were below 1 kU_A/L. Seven of the negative children had responses to one or more peanut-related components in the microarray just below cut-off (data not shown).

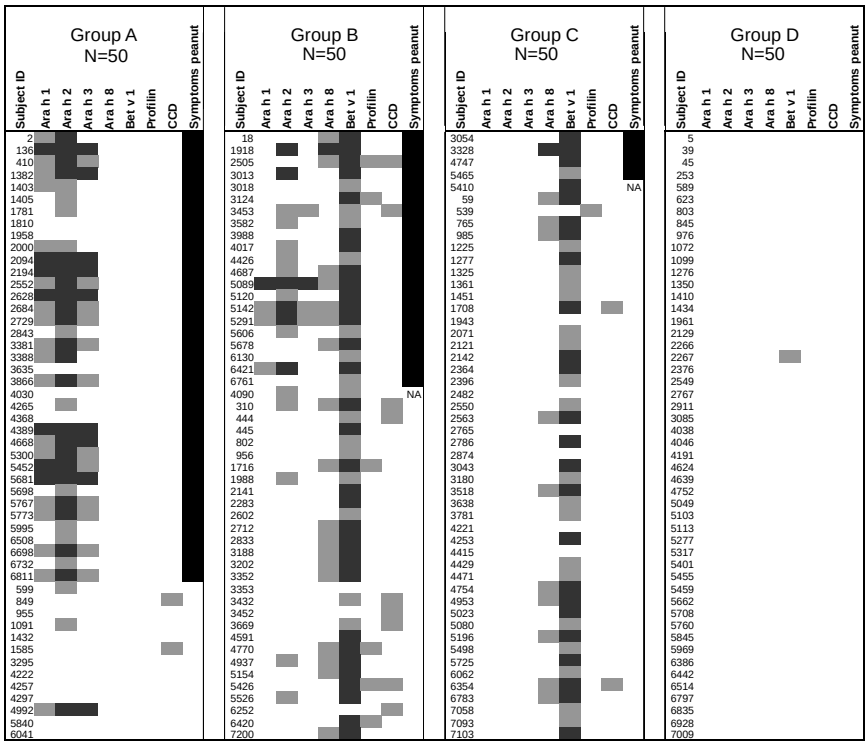

Figure 10. IgE reactivity pattern to individual peanut allergens, Bet v 1, profilin and cross-reactive carbohydrate determinant (CCD) measured in a microarray-based assay.
Group A; children sensitized to peanut and birch pollen, group B; children sensitized to peanut but not birch pollen, group C; children sensitized to birch pollen but not peanut, group D; children sensitized neither to peanut nor to birch pollen. The intensity of the IgE antibody binding is indicated as light grey (medium/low binding, >400-600 FU depending on allergen) and dark grey squares (high binding, >5000 FU). Subjects with self-reported peanut allergy are marked with a black square in the symptom column. NA, information not available. *Asarnoj et al, Allergy 2010; 65: 1189–1195.*

4.3.1.2 Reported symptoms to peanut within the four groups and in relation to peanut allergen components

Symptoms to peanut were more common in peanut-sensitized children without concomitant birch pollen sensitization (group A: 74%, 95% CI 60%-85%) than in children sensitized to both peanut and birch pollen: (group B: 43%, 95% CI 29%-58%) Figure 10.

IgE reactivity to Ara h 2 was found in 73% of the children with reported allergic symptoms to peanut. Only seven of the peanut tolerant children (5%) had IgE antibodies to Ara h 2, all but one with low/moderate IgE reactivity (<5000 FU)(Figure 10).
Symptoms to peanut were reported among the 46 children (group A and B) with IgE reactivity to Ara h 1, 2 or 3 but not Ara h 8 and the 23 children in group B and C with IgE reactivity to Ara h 8 only and not to Ara h 1, 2 or 3 (Figure 11). Eighty-seven percent of those with Ara h 1, 2 or 3 IgE reactivity reported any symptom to peanut,

whereas only 18% of those with IgE reactivity to Ara h 8 reported such symptoms (P<0.001). The differences were significant only for any symptoms as well as upper and lower respiratory symptoms, possibly due to the limited number of children (Figure 11). Interestingly, sensitization to Ara h 1 or Ara h 3 *in addition* to Ara h 2 as compared to children sensitized to Ara h 2 only was associated with several symptoms (97% vs 70%) and more respiratory symptoms (50% vs 9%) (p=0.016 and p=0.002, respectively).

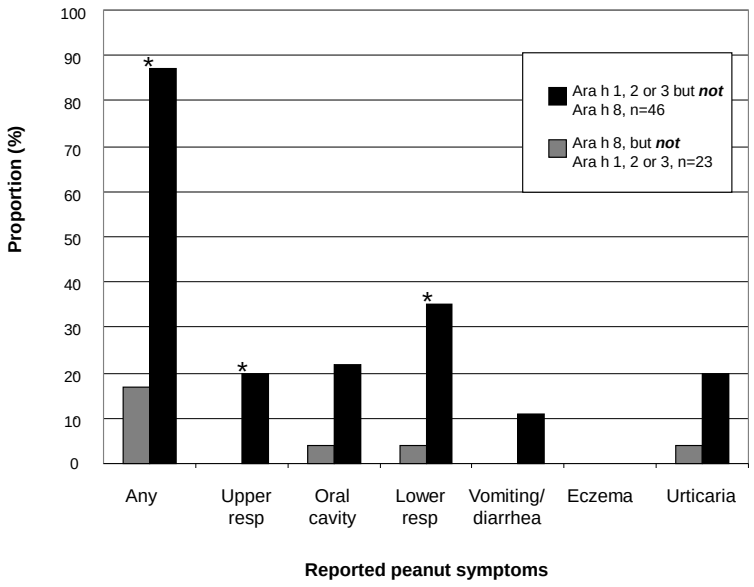

Figure 11**.** Parental reported symptoms to peanut during the last 12 months among children sensitized *either* to at least one of Ara h 1, h 2 or h 3 (n=46, black bars) *or* to Ara h 8 but not to Ara h 1, h 2 or h 3 (n=23, grey bars). *Asarnoj et al, Allergy 2010; 65: 1189–1195.*

IgE reactivity to profilin (Bet v 2 and Phl p 12), the major grass allergen components (Phl p 1, 4 and 5), grass extract or CCD was not associated with reported symptoms to peanut (data not shown).

4.4 PEANUT COMPONENT ARA H 8 SENSITIZATION AND TOLERANCE TO PEANUT (PAPER IV-V)

Paper IV reports a case of anaphylaxis grade II in a 15 year old boy.
This patient was allergic to pollen and furred pets with symptoms of rhino-conjunctivitis, a doctor diagnosed asthma and atopic eczema. Following a positive allergy test before school age, he has refrained from consumption of tree nuts and peanuts. He had no history of anaphylaxis. He was IgE-tested nine months prior to challenge and fulfilled our inclusion criteria for the pre-study, i.e. exhibited IgE

antibody levels to Ara h 1, Ara h 2 and Ara h 3 < 0.35 kU_A/L and to Ara h 8 > 0.35 kU_A/L.
At the open challenge, he exhibited oral itch, stomach pain, vomiting, diarrhea and developed a lower respiratory obstruction, but without reduction in SaO2 (99%). He became increasingly anxious, fatigue and restless. Blood pressure and heart rate were stable and normal. He was given oral antihistamine, adrenaline i.m., intravenous fluid, betametason i.v. and salbutamol inhalation. He was hospitalized and recovered in a couple of hours.
IgE testing of a blood sample taken at the time of challenge showed a doubling of IgE antibody concentration to peanut as compared to a sample drawn 9 months earlier. In addition, barely detectable levels of IgE antibody to Ara h 2 and Ara h 9 were noted, however, not at all accounting for the rise in IgE to the peanut allergen extract. Analysis of IgE to Ara h 6 in a blood sample drawn two months after the peanut challenge revealed a clear sensitization to Ara h 6 (24 kU_A/L), despite a very modest level of IgE to Ara h 2 (0.12 kU_A/L).

A flowchart of the 160 included children and challenges in paper V is illustrated in Figure 12. Eighty-two children were peanut consumers and were not invited for challenge. Sixty two children were invited for challenge among which 44 had never tasted peanuts, 12 had previously experienced reactions to peanut and 6 had eaten peanuts earlier in life without symptoms, but had been advised to avoid peanuts because of previous allergy test results. No children with anaphylaxis gr II-III after exposure to peanuts were found. It is noteworthy that the children who ate peanuts and were therefore not challenged, did not exhibit lower IgE levels to peanut or to Ara h 8 than the group of challenged children; rather the contrary (median peanut IgE among challenged/ not challenged was 1.3 vs 1.6 kU_A/L and corresponding Ara h 8 IgE among challenged/ not challenged was 9.4 vs 14 kU_A/L, and with no statistically significant differences).

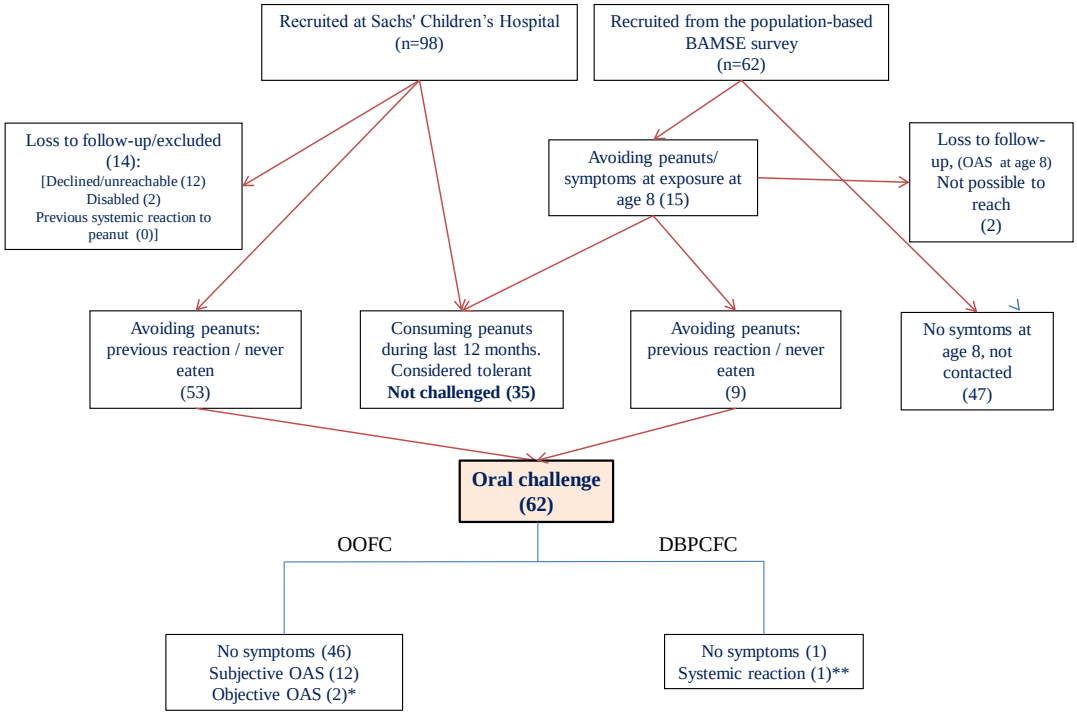

Figure 12. Flow chart of 160 children, recruited from two different sources, initially sensitized to Ara h 8 ≥0.35 kU/L but not to Ara h 1, Ara h 2 and Ara h3 <0.35 kU/L, of whom 62 underwent oral challenge.

* Urticaria in perioral eczema/ redness in soft palate; ** See Table 2.

4.4.1.1 Open oral challenge (OOFC)

Only 14 OOFC children (23%) reported oral cavity or pharyngeal itch (OAS) after challenge, but in only two of these children symptoms could be verified objectively, Figure 12. Interestingly, the OAS occurred at the first or second dose, ceased spontaneously and did not occur at the following dose steps. There was no association between IgE-levels to peanut extract, to Ara h 8 or to birch pollen in relation to OAS symptoms. There were no statistically significant differences in age, sex or other allergy-related disease between children with and without OAS.

4.4.1.2 DBPCFC

In two children, DBPCFC with peanut was carried out since they both had had suspected systemic reactions/anaphylaxis grade I to peanut previously; one within the study and another before the study. One study subject ("subject 1") was accidently challenged during birch pollen season when having symptoms to birch pollen. At challenge he reacted with worsening of his conjunctivitis and rhinitis as well as subjective breathing difficulties. At re-challenge (DBPCFC), 6 months later and outside birch pollen season, he passed the DBPCFC. His IgE to peanut extract and peanut components (including Ara h 6 and Ara h 9 <0.35kU_A/L) were almost unchanged at challenge as compared to time of inclusion.

The second study subject ("subject 2"), a nine-year-old boy, had been admitted to an emergency room with anaphylaxis grade I after having had snacks containing peanut five years prior to our study. He performed a DBPCFC and reacted to the last dose with lip swelling, stomach cramping and objective tiredness, Table 2. At the time of the challenge, his IgE to peanut extract was 8.8 kUA/L, but nine months prior to challenge his IgE to peanut extract had been 1.5 kUA/L. All peanut components were unchanged, Table 2.

Table 2. Characteristics, IgE levels and symptoms of a 9-year-old boy ("subject *2*") who underwent DBPCFC due to a previous suspected anaphylaxis grade I.

Challenge	**DBPCFC**						
Sex/age yrs	Boy/9						
Sachs' patient/BAMSE	Sachs						
Tasted peanut	Yes						
	f13	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9
Peanut sIgE prior inclusion	<0.35	<0.35	<0.35	<0.35	n.d.	1.7	n.d.
Peanut sIgE 9 months prior challenge	1.5	<0.35	<0.35	<0.35	0.45	14	<0.35
Peanut sIgE at re analysis 1 month prior challenge	8.8	<0.35	<0.35	<0.35	0.45	14	n.d.
Symptoms at previous exposure	Age 4 years. Oral tingling, unilateral conjunctival swelling, stomach pain, tiredness						
Symptoms at challenge	Lip swelling, stomach cramping, tiredness						

4.4.1.3 IgE to peanut allergen components at challenge

No blood was drawn from three children at challenge, all of them without symptoms at challenge. Serum from 59 (95%) of the challenged children was re-analysed for IgE to peanut and birch pollen extracts as well as to peanut components. All children still exhibited IgE to Ara h 1, h 2 or h 3 <0.35 kU_A/L, despite the fact that 9.7 months had passed in average between identification and challenge. Two children had Ara h 6-IgE >0.35 kUA/L: one child had IgE to Ara h 6 of 0.73 kU_A/L and passed the challenge. Another child with IgE to Ara h 6 of 0.45 kU_A/L failed (Table 2) and developed a systemic reaction (see above). In none of the patients neither CCD- nor Ara h 9 (LTP) IgE levels were associated with any symptoms at challenge (data not shown).

4.5 ADDITIONAL RESULTS

4.5.1 Comparison of component analyses results: Microarray and ImmunoCAP

In November 2009, all available sera (n=185) of peanut sensitized children of the BAMSE cohort (n=195) from the eight year follow-up were analysed for IgE to peanut allergen components Ara h 1, h 2, h 3, h 8 and h 9 (lipid transfer protein) which at this time all was commercially available. The ImmunoCAP method was used. Comparison of these results with component IgE results from the experimental microarray, performed in 100 of the 195 sera in December 2007, is presented in Figure 13.

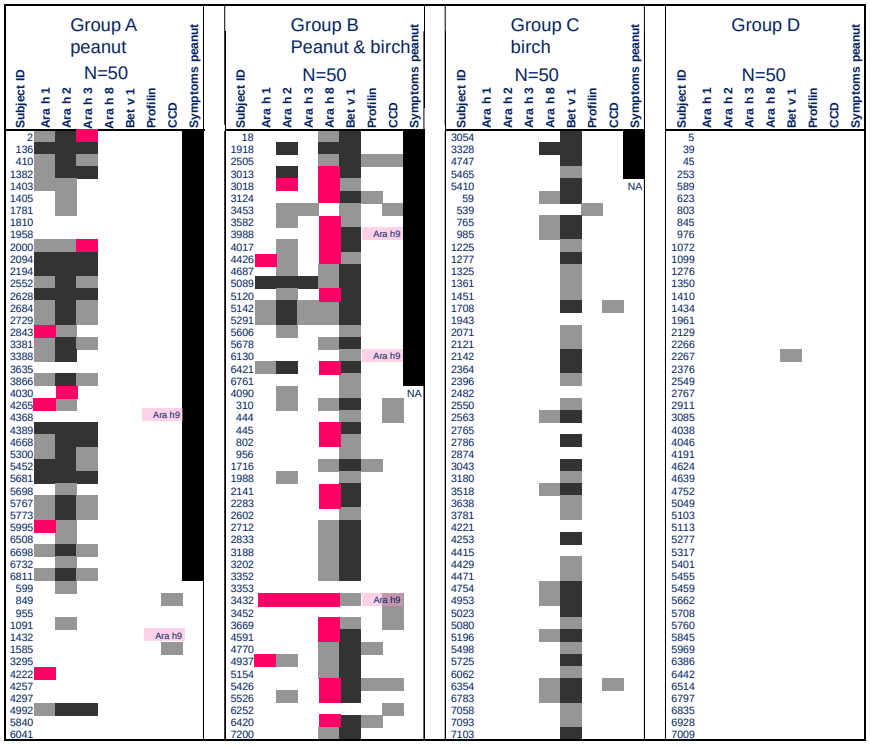

Figure 13. Modified Figure 10, paper III, page 26. Detection of IgE with the ImmunoCAP method of children sensitized to peanut extract, but negative to peanut allergen components by using the microarray technique (red and pink).

Sixty-six of the 100 sera analysed with both methods showed corresponding results. Nineteen sera negative (<600 FU) in the microarray assay for Ara h 8 were positive with the ImmunoCAP for Ara h 8. The median ImmunoCAP Ara h 8 IgE was 4.7 kU_A/L (range 0.52-46) among those initially negative for Ara h 8 with the microarray as compared to 33 kU_A/L (range 8.9-99) among the microarray positive samples. The peanut LTP component Ara h 9 was not analysed with the microarray. Instead, a peanut LTP proxy, the peach LTP (Pru p 3) was used and was negative in all 100 peanut-positive samples. When the ImmunoCAP method was used, five sera (5%) showed IgE to Ara h 9 (range 0.36-1.0 kU_A/L), three of these children reported peanut symptoms or peanut exclusion, no systemic symptoms were indicated. Taking the results from both ImmunoCAP and the experimental microarray together, IgE to peanut allergen

components could still not be detected in 13 (one missing sample in ImmunoCAP) of the 100 originally peanut extract sensitized children (as compared to 25 sera of peanut sensitized children but without reactivity to to peanut components when using the microarray). Thus, some peanut allergen components seem to be lacking. However, median peanut IgE level among in the 13 sera was low; 0.58 kU_A/L (range 0.36-3.3 kU_A/L). Three of these children reported symptoms at exposure to peanut, but in none of the children the symptoms were systemic.

Of the 185 peanut extract sensitized children (>0.35 kU_A/L), peanut component allergen IgE analyses with ImmunoCAP showed that in 32 (17%) children, IgE to Ara h 1, h 2, h 3, h 8 and h 9 was below 0.35 kU_A/L. Among these children median IgE to peanut extract was 0.8 (range 0.41-5.4) kU_A/L. Seven of them reported any symptom to peanut or exclusion of peanuts, no report of systemic symptoms except one who reported eczema after peanut exposure. Of the 32 children, 15 had IgE to at least one of the tested peanut allergen components between 0.1 and 0.34 kU_A/L or IgE to CCD/profilin in the microarray. For the remaining 17, no detectable IgE to any commercially available peanut allergen component was found, indicating that all peanut allergen components are not available for IgE testing. However, 13 of these 17 children were peanut tolerant. The other four did not indicate any systemic reactions.

4.5.2 Children with reported systemic reactions to peanut in Paper III sensitized to Ara h 8 only

In study III, two eight year olds sensitized to Ara h 8 and not to Ara h 1-h 3 reported systemic reaction to peanut at exposure (breathing difficulties and urticaria, respectively). Both of them were included in study V and had an oral peanut challenge performed about seven years after the eight years of age follow up. They both passed the challenge without any symptoms, not even having symptoms of OAS, Figure 14. At retesting before the challenge the children had the same sensitization profile with IgE only to Ara h 8 and not to Ara h 1, h 2 or h 3.

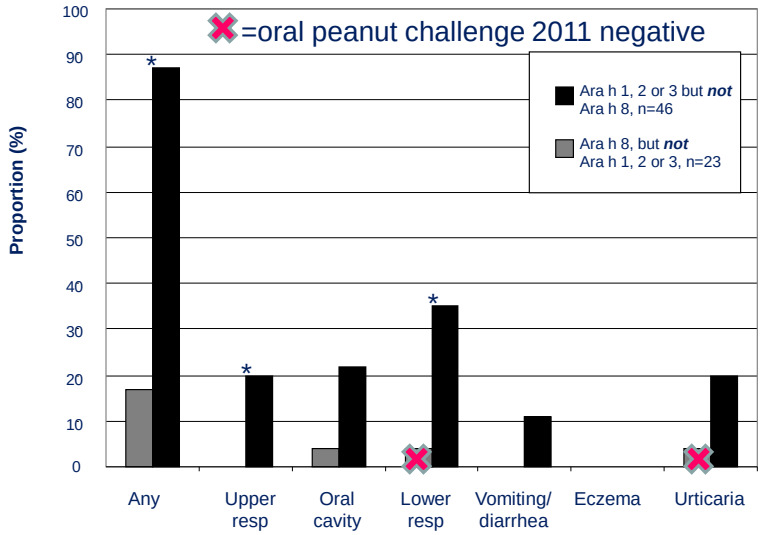

Figure 14**.** Modified Figure 11, paper III, page 27. Parental reported symptoms to peanut during the last 12 months among children sensitized *either* to at least one of Ara h 1, 2 or 3 (n=46, black bars) *or* to Ara h 8 (n=23, grey bars) at eight years of age. The children reporting systemic reactions to peanut were seven years later orally challenged with peanut (study V) without any symptoms.

4.5.3 IgE Immunoblotting (Paper V)

The boy with anaphylaxis grade I in paper V had unchanged IgE levels to known peanut components despite increasing levels of IgE to peanut extract (1.5 to 8.8 kU_A/L). Thermo Fisher Scientific helped us to perform an immunoblot (or Western blot) on the boy's serum outside the study. Peanut proteins were first separated according to size by gel electrophoresis and then transferred horizontally to a flat membrane where the proteins were available for detection by IgE antibodies present in the boy's serum. The immunoblotting showed intense IgE binding to one protein band at 35 and faint binding to a double band at about 70 kDa, but no binding to smaller protein molecules (Ara h 2, h 6, h 8-9). The IgE binding proteins observed in the immunoblot analysis did not share epitopes with rAra h 1 or rAra h 3 and are of unknown identity.

5 DISCUSSION

5.1 THE SENSITIZATION PROCESS OVER TIME

Our results of the prevalence of sensitization on inhalant allergens at four (15%) and eight (25%) years of age in paper I are in line with other studies[107-110]. For reasons of exposures, allergen patterns of sensitization will be different in different regions around the world. For example among trees, birch pollen sensitization dominates in Northern Europe, whereas sensitization to hazel pollen dominates in Central/Western Europe and olive pollen sensitization in the Mediterranean countries[111, 112]. The sensitization prevalence to house dust mites (HDM) was low in our study (1.8 % and 4.1% at four and eight years of age, respectively) as compared to others. For example Kjaer Formsgaard et al reported 7.4% sensitized among six year-olds in their population based birth cohort ("DARC") from Odense Denmark[21]. This difference in sensitization rate to mites between the DARC and the BAMSE study is probably due to low exposure to HDM in the Stockholm area[113, 114].

Several studies have been designed to describe the natural course of sensitization to common inhalant allergens in the population[21, 107, 115, 116]. For all studies a dynamic process of allergic sensitization seem to be common over time. Grass sensitization is the dominant sensitizing allergen among inhalants across Europe in contrast to birch sensitization which dominated in our study. As for the other allergens tested for in our study, similarities in sensitization patterns were seen with other studies: the prevalence of sensitization to inhalant allergens increases during childhood whereas reduction in IgE levels below detection level to inhalant allergens occurs rarely and when present this is mostly found when IgE-levels were low at previous follow up[115, 116].

Between four and eight years of age, occurrence of specific IgE to the most common inhalant allergens almost doubled in our cohort. The relative increase in the proportion of sensitized children was larger for timothy and dog as compared to birch and cat. Both timothy and dog sensitization seemed to catch up in prevalence and was rather similar compared to prevalence of sensitization to birch pollen and cat dander at eight years of age. To our knowledge this has not been described before. To our surprise, birch pollen sensitization at eight years of age was strongly associated with sensitization to peanut at eight years of age (OR 24.2) and peanut sensitization at eight years of age was more common as compared to other similar population based studies[40, 41]. In paper I, the association between sensitization to birch pollen and peanut was not mentioned since we did not have any fair explanation and since the finding could be more of by chance character at the time for this study. However, since these results were rather intriguing a decision was taken to go into depth with our data, since we also became aware of the paper by Mittag et al on Bet v 1 and Ara h 8 cross reactions, which actually resulted in the remaining part of this thesis[51].

5.2 FROM SPT AND IGE OF ALLERGEN EXTRACTS TO CRD

In late 2006/early 2007, at the start of studies II and III, the field of CRD in clinical care was still quite unknown and not in use. The best available tool for allergy diagnostic purposes was quantification of SPT or serum IgE results in order to predict the likelihood of a clinically relevant allergy[42, 61, 65, 67, 117]. Since more than half a century it

has been known that allergic rhinitis during springtime is associated with oral cavity symptoms to some fruits and tree nuts[70, 71]. Cross-reactions between pollens and food items is common and causes oral allergy syndrome (OAS)[60]. Such cross-reactions were actually clinically described for birch pollen and peanut already 1982[54] and at a molecular level in 2004-2005[51, 118]. However, in review articles on food allergens or OAS at the study start such cross reactions were either not mentioned or considered as representing "true" peanut allergy and not only OAS due to cross reactions between birch pollen and peanut[79, 118, 119].

In Paper II, we could for the first time demonstrate that there was a significant difference in frequency of reported peanut symptoms in eight year olds sensitized to A) both to peanut and birch pollen or B) to peanut, but not to birch pollen (40% and 76% report of symptoms, respectively, p= 0.002). Furthermore, we could show that symptoms to peanut in the former group (peanut and birch pollen sensitized) were milder than in the latter group (only peanut sensitized). The most plausible reason for this was the cross-reactivity between allergens in birch pollen and peanut, since peanut allergen extract contains Ara h 8, a PR-10 allergen, which is homologous to the major birch pollen Bet v 1. Possibly, Ara h 5 which is a profilin and therefore homologous to the birch pollen profilin Bet v 2 may have played a role as well in this context. Both Ara h 8 and Ara h 5 have been characterized by others[51, 87]. Other studies on sensitization to peanut and other pollens (grass) have also been found to be in line with our findings of peanut tolerance in peanut and birch pollen co-sensitized individuals[41]. Furthermore, there are studies which describe similar associations between birch pollen and other food items, e.g. allergens of hazelnut and birch pollen[54, 71, 120].

In paper II we found that IgE levels to peanut were lower in the "peanut-and-birch" group, compared to the "peanut only" group (geometric mean 4.3 kU_A/L and 12 kU_A/L, respectively, p=0.0032). Low IgE to peanut in a population based sample may reflect cross-reactivity to other allergens of plant origin, in particular that of pollen. In cases of "true" peanut allergy, higher IgE levels to peanut are usually seen[67]. However, this may partially be age dependent, i.e. very young children with anaphylaxis to peanut may still have low IgE to peanut (personal communication Magnus Wickman and Caroline Nilsson).

In Paper III we wanted to further examine the relationship between cross sensitization between pollen and peanuts. The presence of relevant allergen components explaining the differences in symptoms between the sensitization groups described in Paper II was investigated. In 2007, 200 blood samples, representing four different sensitization patterns regarding peanut and birch pollen, were analysed in an experimental microarray[121]. We found that 83% of those with IgE reactivity to the peanut component Ara h 2 also reported allergic symptoms to peanut, compared to children exclusively sensitized to Ara h 8 where peanut symptoms were reported in 18% only. These results are in line with other studies, showing the importance of Ara h 2 IgE in peanut allergy[122-125] . In addition, the symptoms in the latter group of 23 Ara h 8 sensitized children were milder, although two children reported symptoms likely of systemic origin (lower respiratory symptoms and urticaria, Figures 11 and 14) which is discussed further below in 5.4.

Since the participating children in Paper II and III were not challenged, we were not able to draw very firm conclusions of the clinical relevance of Ara h 8 sensitization. Therefore, we decided to perform a study in order to evaluate the risk of systemic reactions for individuals with isolated Ara h 8 sensitization (Paper V). At the time for preparation of this study, we learned that IgE to the 2S albumin Ara h 6 may occur even in the absence of IgE to Ara h 2, another 2S albumin, and most likely causing a severe reaction to peanut (Paper IV, case report). Isolated Ara h 6 sensitization should be rare since the two 2S albumins Ara h 2 and Ara h 6 are homologues[87, 126]. As a result of this finding, we decided to perform extended analysis of peanut IgE, including also Ara h 6 in study V.

143 (99.3%) of the 144 children evaluated in study V were tolerant to peanut or exhibited mild OAS, mainly with subjective symptoms from the oral cavity that disappeared spontaneously during the challenge procedure. One boy had a DBPCFC because of a suspected systemic reaction at an open challenge during birch pollen season and with symptoms of rhino-conjunctivitis at challenge. However, apart from pollen season he passed a DBPCFC to peanut. This boy's first challenge during pollen season failed, which indicates there is an elevated risk of peanut reaction at challenge for individuals with on-going allergic symptoms. This is also in accordance with the observation in a recent paper from our group where we could show that anaphylaxis to foods is more common during pollen season among pollen allergic individuals[127]. The only boy who had a suspected systematic reaction within the study had a previous history of possible systemic reaction as well as a slightly elevated Ara h 6 IgE (0.45 kU_A/L). Immunoblot analysis revealed that an unknown protein of about 35 kDa accounted for most of the binding of peanut-reactive IgE. We believe it is more likely that the symptomatic reaction occurred as a result of sensitization to this protein of unknown identity than to Ara h 6 IgE, which only accounted for a small proportion (0.45 kU_A/L) of the IgE response to peanut extract (8.8 kU_A/L).

5.3 PEANUT ALLERGENS

The diagnostic utility of molecular allergology or component resolved diagnostics (CRD)[128] has developed rapidly since this thesis project started in 2006. Below is a summary of gained knowledge from 2006 to date (2012) with respect to peanut allergens and allergenicity in relation to results from our studies. A list of officially recognized peanut allergens is established by International Union of Immunological Societies (IUIS) Nomenclature Subcommittee and available at www.allergen.org. Eleven different peanut allergen components named Ara h 1 through Ara h 11 are identified in total by April 2012.

5.3.1 Peanut allergen components in relation to our results

5.3.1.1 Ara h 8

Birch pollen Bet v 1 homologous components (members of the PR-10 protein family) are present in many plant derived foods, for example Ara h 8 in peanut, Gly m 4 in soy[129, 130], Cor a 1 in hazelnut[131, 132] and Mal d 1 in apple[133, 134]. PR-10 mediated reactions to these foods seem to differ quite considerably in frequency and severity. Severe reactions derived from Ara h 8 or Mal d 1 have to our knowledge not been

described so far, whereas systemic reactions to soy are documented in patients with isolated Gly m 4 sensitization[129, 135, 136]. Significant reactions in patients with Cor a 1 sensitization without sensitization to hazelnut storage proteins or LTP (Cor a 8) have been described in a minority of patients, but there are too few studies to draw firm conclusions on Cor a 1 and risk of systemic reactions[131]. The Ara h 8 protein is present in very low amount in peanuts and is known to have low stability to gastric digestion and heat [51]. This is likely the explanation of our findings of symptoms only from the oral cavity among those with isolated Ara h 8 sensitization. The low stability also suggests that Ara h 8 sensitization is primarily driven by birch pollen cross-reactivity and not through passage of the gastrointestinal tract. Possibly, roasting of peanuts partly destroys the Ara h 8 protein molecule which explains the low proportion of children who react at exposure to commercially available peanuts which are roasted in our part of the world. However, if a larger amount of peanuts would be ingested rather rapidly, and Ara h 8 only become partly degraded, a systemic reaction may theoretically occur in analogy with described reactions from the soy protein Gly m 4[135, 136] Mittag et al characterized Ara h 8 in 2004 and performed oral challenges in peanut sensitized individuals, sensitized to not only Ara h 8, but also to Ara h 1-3. In their paper they suggest that severe reactions caused by Ara h 8 may occur[51]. However, it is evident from their paper that all the six patients with isolated Ara h 8 sensitization reacted only with OAS at challenge, except one patient who also exhibited dermal flush.

5.3.1.2 Ara h 1, h 2, h 3 and h 6

The 2S albumin peanut component Ara h 2 sensitization was in Paper III the most important marker of genuine peanut allergy, as in several other studies[37, 122-125, 137]. The Ara h 2 protein is stable to heating and gastric digestion[138-143]. In our study the strong association to peanut symptoms was found in particular in children with Ara h 2 sensitization in combination with sensitization to Ara h 1 and/or Ara h 3. IgE binding to increasing number of peanut allergens has shown to be associated with more severe reactions to peanut[144]. Alternatively, concomitant sensitization to Ara h 2 and Ara h 1/h 3 may solely be a consequence of high IgE levels to Ara h 2; IgE levels to Ara h 1 and Ara h 3 generally are lower than to Ara h 2[101] and could both be below detection limit in patients with low IgE reactivity to Ara h 2. High IgE-levels to Ara h 2 are associated with increased risk of peanut allergic symptoms[37, 101]. Quantitative IgE levels were not available in Paper III since the microarray method is only semi-quantitative. However, all children with strong IgE response to Ara h 2 in the microarray (>5000 FU) were also sensitized to Ara h 1 and/or Ara h 3, Figure 10. Ara h 1 and Ara h 3 sensitization *per se* are of less diagnostic value for peanut allergy compared to Ara h 2[123, 124] and in our study Ara h 1 and Ara h 3 IgE were only found in combination with Ara h 2 sensitization.

The Ara h 2 homologue 2S albumin Ara h 6[87] has recently been found to be a relevant peanut allergen. Like Ara h 2 it has high stability to digestion and heat as well as high degranulation capacity *in vitro* [138-143, 145]. Ara h 2 and Ara h 6 are extensively cross-reactive and Ara h 6 IgE is thought to be found only in Ara h 2 sensitized individuals[87, 137, 146]. However, in Paper IV, we concluded that Ara h 6 sensitization may occur even in the absence of Ara h 2 sensitization and also may cause a severe reaction to peanut at exposure.

5.3.1.3 Other peanut allergens: Ara h 5(profilin), Ara h 9 (LTP) and CCD

Peanut profilin Ara h 5, homologous with birch Bet v 2 profilin, is considered as a minor peanut allergen, and may serve as a marker of any profilin sensitization from different plant sources[75]. In Paper III, Ara h 5 was not measured since it was not available. Instead Ara h 5 was represented by birch profilin Bet v 2 and timothy profilin Phl p 12. Such IgE reactivity was seen in six of the 100 peanut sensitized children, all six were also birch sensitized and none of them reported systemic symptoms to peanut. Peanut LTP Ara h 9 was identified by Krause et al in 2009[147]. LTP proteins are known to be highly stable to digestion and heating[148, 149]. Ara h 9 is an important and clinically relevant peanut allergen in the Mediterranean area, but likely not in Central and Northern Europe where sensitization is rare[123, 150, 151]. Our findings in Paper III are in line with these geographical differences: in the 100 peanut sensitized Swedish children, the microarray method did not recognize any LTP-sensitized individual (Pru p 3) although the additional ImmunoCAP analysis identified species specific IgE to Ara h 9 (>0.35 kU_A/L) in five children. Three of them reported exclusion of peanuts from diet or local symptoms to peanut and there was no association to systemic reactions. In Paper V, Ara h 9 was determined in 59 children who were peanut challenged. Only two of these children were tested positive (>0.35 kU_A/L) for Ara h 9 IgE; one of them (Ara h 9 IgE 4.1 kU_A/L) reported oral cavity symptoms of the initial doses at the oral challenge which ceased at the last doses, the other was tolerant (Ara h 9 IgE 8.3 kU_A/L).
Cross-reactive carbohydrate determinants (CCD) IgE is induced by pollen sensitization and considered to contribute to clinically irrelevant peanut sensitization[152]. Our studies support that postulation; 11% and 10% of tested children in Paper III and V, respectively were CCD sensitized. None of these children had systemic symptoms except for one child in Paper III, who also was Ara h 2 sensitized.

5.3.2 Clinical utility of peanut component IgE testing

There are now numerous studies showing that Ara h 2 IgE provides good accuracy as a diagnostic tool for peanut allergy in many areas[37, 122-125]. However, lack of Ara h 2 sensitization can probably not be used to predict peanut tolerance worldwide, since other allergenic molecules, such as Ara h 9, may be of clinical importance in certain areas, e.g. Spain[151]. Hence, increasing IgE levels to whole peanut extract without increasing component IgE levels of those which are available today should prompt caution. Detection of isolated IgE to Ara h 8 in a peanut sensitized individual may primarily serve as a valuable pedagogic diagnostic instrument for information of the clinical relevancy of peanut sensitization in birch allergic patients.

The problem with sensitization without symptoms or sensitization without previous exposure has resulted in considerable anxiety and fear of severe reactions to peanut in otherwise rather healthy individuals. Food allergy has shown to have a negative impact on quality of life[49, 50, 153]. With the use of CRD, we are now hopefully heading towards less restrictive advices on elimination of foods from the diet in a substantial number of birch pollen allergic patients. The difficulties to disentangle harmless reactions from harmful will hopefully be reduced by using allergen component testing.

However, there may be allergen components not yet characterized. Their importance from a clinical point of view needs to be shown. In Paper III, we noted that out of 100 peanut sensitized children (IgE to peanut extract>0.35 kU_A/L) we could not pinpoint the sensitizing peanut allergen in 25 sera. After re-analysing the samples with more sensitive ImmunoCAP, commercially available from autumn 2007 (Magnus Wickman personal communication), 13 of these 100 sera were still lacking explanatory sensitizing peanut components (one missing sample) and none of these children reported systemic reactions to peanut.

5.4 STRENGTHS AND WEAKNESSES OF THE PRESENT INVESTIGATIONS

In the studies including children from the BAMSE cohort (Paper I-III and V), the population based design enables us to generalize our findings to the paediatric population in corresponding ages, at least in the Stockholm area. The longitudinal approach makes it possible to study the sensitization process and development of symptoms to peanuts over time in the same individuals. The large number of participants and high response rates at all follow ups are unique. The proportion of smokers was lower amongst parents in included families as compared to non-responders initially of the study[96]. Nevertheless, if this lower participation rate among smokers would influence the frequency of allergic diseases, it would probably lead to underestimations rather than overestimations of disease, since smoking is a known risk factor for the development asthma[154-156].

The ImmunoCAP specific IgE assay is the most commonly used assay for analysis of serum levels of IgE antibodies and it is approved by the US Food and Drug Administration[4]. The method is standardized. When possible, this assay was used for analysing circulating IgE levels in our studies. In Paper III, specific IgE analysis to peanut components was not available on ImmunoCAP, instead an experimental microarray assay was used. The advantage of this assay was the ability to analyse IgE antibodies to a large number of allergen components with a very small volume of serum (30µl). The drawback of using the experimental microarray assay was reduced performance in sensitivity when it comes to detection of IgE to peanut components, especially IgE to Ara h 8, see above 4.5.1. The reason for this may be that the choice of cut off-level was set relatively high (600 FU) due to higher fluorescent background than for instance for Bet v 1 (cut off level 500 FU). Another speculative explanation could be that the fragile protein structure of Ara h 8 may be better preserved when coupled to the ImmunoCAP solid phase as compared to when spotted to the nitrocellulose membrane used in the microarray assay (personal communication Robert Movérare). Nevertheless, when the peanut-IgE positive serum samples from Paper III were re-analysed with ImmunoCAP it was obvious that the systematic measurement errors in the microarray diluted the results and not the opposite, since most of the additionally detected IgE reactivities were to Ara h 8 in asymptomatic individuals (Figure 13).

In Paper II and III, data on peanut symptoms were derived from questionnaires and not from observed oral food challenges which are considered more objective[35]. Self-reported symptoms or - as in our studies - parental reported symptoms, are known to be

over reported[157], and hence most probably would dilute the associations of peanut tolerance and concomitant birch sensitization/isolated Ara h 8 sensitization. In our challenge study (Paper V), we were able partly to evaluate this possibility of over reporting. Thus, some children were invited for oral peanut challenge after several years had passed since they were identified as possibly Ara h 8 sensitized. The two children with isolated Ara h 8 sensitization who reported systemic symptoms from peanut in Paper III (Figures 11 and 14) and in the eight years of age questionnaire were challenged within the study V at the age of 14 and 17 years, respectively, and were found to be peanut tolerant at oral challenge. Of course, we cannot rule out that these children had grown out of the clinical peanut allergy even if this is unlikely since the IgE levels and pattern of sensitization to peanut was identical.

Since our main aim was to evaluate the risk of systemic peanut reactions in children sensitized to Ara h 8, but not Ara h 1, h 2 or h 3 in paper V, we decided that DBPCFC was not needed since systemic reactions are very difficult to fake. Instead, we used an open oral challenge four step procedure in children without history of severe reactions to peanut. This procedure is less time consuming as compared to our standard procedure. Our previous challenges in our clinic in children with isolated Ara h 8 sensitization suggested that there were not many severe reactions to expect. Similar methods have also been used by others[37]. However, when setting up the study we initially had one patient who reacted on our "fast track" oral challenge and at that time with three dosing steps only and with the last dose of 10 g of peanut. Due to this reaction we changed the challenge procedure of the study into a four dosing step design by splitting the last dose of 10 g into two doses of 5 g 20 minutes apart in order to capture severe reactions more rapidly and possibly at a lower dose of peanut.

5.5 CONCLUDING REMARKS AND OUTLOOK

In our first study we have analysed the natural course of sensitization to inhalant allergens between four and eight years of age in a large population based cohort. In short time we intend to analyse IgE data from our on-going sixteen-year follow up of the same cohort. These data will provide unique opportunities to study the sensitizing process in a larger population based sample from pre-school age up to adolescence. There are to date few studies on longitudinal data on sensitization[158]. Sensitization to peanut components in a longitudinal perspective will also be investigated with the aim to describe the natural course of peanut allergen component sensitization.

In Papers II-V we have systematically investigated birch pollen IgE and peanut IgE in relation to symptoms to peanut including the clinical impact of birch homologous peanut component Ara h 8, which seems to indicate peanut tolerance. Ara h 1, h 2 and h 3 seems to indicate genuine peanut allergy. In a near future, we plan to examine associations of IgE levels of Ara h 1 and h 3 in relation to symptom severity and the dependency of IgE to Ara h 1 and h 3 in relation to Ara h 2. Possible, Ara h 1 and h 3 may only serve as proxies for IgE to Ara h 2.

In Paper III and V we failed to identify the sensitizing allergen components responsive for detected IgE in some of the children with sensitization to peanut extract. In paper

III, components explaining the IgE level of the peanut extract were lacking in 13% of the samples analysed all commercially available peanut allergens. However, only three of these 13 children reported symptoms to peanut and no had symptoms of a systemic reaction. The immunoblot which was performed in serum from one study subject with a systemic reaction at challenge indicated major sensitization to a peanut protein of unknown identity (paper V). Future analyses of sera from symptomatic patients with unexplained peanut sensitization may provide evidence on the clinical relevance of this protein.

6 CLINICAL IMPLICATIONS

During the years 2006-2012, the time of doctoral studies for my thesis, there has been a rapid development in the field of allergy diagnosis, in particular diagnosis of food allergy with the introduction of CRD. Our studies have contributed to this development and may have had an impact on clinical routines concerning diagnosing peanut allergy. Many children with peanut sensitization have until recently been advised to avoid peanuts because of risk of severe reactions. A major reason for this was the paper by Sampson H et al where they in 2001 showed that IgE above 15 kU_A/L were associated with a risk of severe reactions. Today, determination of peanut allergen IgE components, in combination with a careful taken clinical history, may help to disentangle children at risk of severe reactions from children with asymptomatic birch pollen cross-reactive sensitization. According to our own data about one third of peanut sensitized children in Sweden should possibly be able to eat peanuts without fear of severe reactions.

From the results of this thesis I have learned that:

- In a population based material of young children sensitization to birch pollen and cat are most prevalent. However, in early school age the prevalence of sensitization to timothy pollen and dog have almost become similar to that of birch pollen and cat which has not been described before.

- Ara h 2 sensitization, especially in combination with sensitization to Ara h 1 and/or Ara h 3, is often associated with clinical peanut allergy.

- Isolated Ara h 8 sensitization seems to indicate peanut tolerance in most cases. In such patients who are avoiding peanuts, this food item may be introduced cautiously at home in order to avoid unexpected severe reactions. The reason for this cautious introduction is the possibility of sensitisation to still not characterized peanut allergen components.

- Ara h 6 cross-reacts extensively with Ara h 2. In rare cases Ara h 6 sensitization may occur in the absence of Ara h 2 sensitization and thereby cause a severe reaction at exposure to peanut. Therefore, it must be very useful to have Ara h 6 commercially available.

- When analysing test results for peanut components, the sum of IgE to peanut components should at least cover the IgE level of the peanut extract. When peanut extract IgE levels are increasing it should be accompanied with a corresponding increase in peanut component IgE. If not, this should be a sign for increased awareness since patients, even though rarely, may be sensitized to peanut allergen components not yet characterized.

- A previous history of a systemic reaction at peanut exposure should always be seriously considered when assessing the risk of a systemic reaction at challenge, irrespective of IgE test results. The reason for this is that all peanut proteins related to IgE-mediated reactions may not yet have been identified and characterized.

- With the use of CRD, we are now hopefully heading towards less restrictive advices on elimination of foods from the diet in a substantial number of birch pollen allergic patients. The difficulties to disentangle harmless reactions from harmful will hopefully be reduced by using allergen component testing.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Allergisjukdomar är en av våra största folksjukdomar och ca 25% av alla barn i Sverige har någon form av allergibesvär. Allergirelaterade sjukdomar har ökat under flera decennier, men ökningen verkar ha planat ut i vissa delar av västvärlden samtidigt som vi ser en ökning i t.ex. Östeuropa och delar av Asien.

Att vara allergisk innebär för de flesta att deras immunförsvar reagerar mot ett i vanliga fall ofarligt ämne. Vid kontakt med ämnet bildas så kallade allergi-antikroppar – Immunoglobulin E (IgE). Denna process benämns sensibilisering. Det allergiframkallande ämnet kallas allergen. Allergenet kan t ex vara proteiner i pollen som når kroppen via inandningsluften eller proteiner i ett födoämne som hamnar i mag-tarmsystemet. En allergisk reaktion triggas igång vid förnyad kontakt med allergenet och visar sig i form av astma, hösnuva, eksem, nässelutslag, svullnader, magbesvär eller i värsta fall allergisk chock som kan vara ett livshotande tillstånd.

Allergier mot mat är vanliga framförallt under barndomen. Beroende på hur denna allergiform definieras så drabbas ca 2%-10 % av befolkningen. Jordnötsallergi är en av de vanligaste formerna av födoämnesallergier och en viktig orsak till allvarliga allergiska reaktioner. Förekomsten i befolkningen varierar mellan 0.6 % och 3 % i olika undersökningar och flera studier pekar på en ökande trend det senaste decenniet.

Korsreaktioner mot födoämnen är mycket vanliga hos personer med pollenallergi. Orsaken är att många födoämnen och pollen innehåller liknande proteinstrukturer. Pollen-IgE-antikroppar korsreagerar mot allergen i födoämnen på grund av likheterna i struktur. Följden blir att den som är pollensensibiliserad också kan vara sensibiliserad mot olika födoämnen. Beroende på hur lika dessa proteiner är varandra så kan symtom uppkomma vid förtäring av födoämnet. I regel blir dessa symtom lindriga och begränsade till munhålan. Serologisk korsreaktion, kanske bättre benämnd korssensibilisering, föreligger ofta utan att några som helst symtom uppkommer vid förtäring av födoämnet i fråga. Det positiva IgE-testet mot födoämnet kan i onödan orsaka oro för att drabbas av allvarliga reaktioner.

Det övergripande syftet med denna avhandling var att studera sensibiliseringsmönster, d.v.s. förekomsten av IgE antikroppar, mot luftburna allergen under barndomen. Dessutom att studera björkpollen- och jordnöts-antikroppar samt antikroppar mot olika strukturer i jordnöt i relation till symtom på jordnötsallergi.

Studierna baseras till största delen på material från BAMSE-studien (Barn, Allergi, Miljö i Stockholm, en Epidemiologisk studie); en undersökning med fokus på att studera utveckling av allergisjukdomar innefattande drygt 4000 barn som följs sedan födseln i början av 1990-talet. Den sista delstudien i avhandlingen är utförd på Sachsska Barnsjukhuset och inkluderade 98 barn från allergimottagningen samt 62 barn från BAMSE-studien.

I den första studien beskrevs sensibilisering mot allergiframkallande ämnen i luften, främst pollen och pälsdjur, mellan fyra och åtta års ålder. Vi konstaterade att det är en mycket dynamisk process där andelen sensibiliserade barn ökade från 15 % till 25 %

mellan fyra och åtta års ålder samt att antikroppar mot björkpollen och katt dominerade. Ökningen i förekomst av sensibilisering mot timotej och hund var dock relativt sett större och verkade ”knappa in” på förekomsten av sensibilisering för björkpollen och katt.

I studie 2 visade vi att åttaåringar som var sensibiliserade mot *både* björkpollen och jordnöt i mindre utsträckning uppgav symtom vid förtäring av jordnötter jämfört med åttaåringar som var sensibiliserade mot jordnöt men *inte* mot björkpollen. Detta tvärtemot vad man skulle tro eftersom IgE mot flera olika ämnen brukar resultera i högre förekomst av allergisymtom.

I den tredje studien ville vi titta närmare på orsakerna till fynden i studie 2 och analyserade IgE-allergena komponenter mot pollen och jordnöt. Allergena komponenter är de olika molekyler, framför allt proteinmolekyler i t.ex. ett födoämne som kan ge upphov till allergiantikroppar (IgE). Nomenklaturen vid komponentanalys bygger på det latinska namnet för växten eller djuret i fråga samt i vilken ordning proteinet karakteriserades. För jordnöt (Arachis hypogaea) får allergena komponenter de tre första bokstäverna från familjenamnet (Ara) och den första bokstaven från artnamnet (h). Allergisk sensibilisering för jordnötskomponenterna Ara h 1, Ara h 2 och Ara h 3 anses vara de proteinmolekyler som ger upphov till ”äkta” jordnötsallergi medan sensibilisering för den björkpollen-liknande komponenten Ara h 8 ses vid samtidig sensibilisering för björkpollen p.g.a. den serologiska korsreaktionen mellan Ara h 8 och huvudallergenet i björkpollen. Vi kunde konstatera att Ara h 2-IgE var starkast kopplad till symtom mot jordnöt, särskilt i kombination med IgE mot Ara h 1 och/eller Ara h 3. De barn som var sensibiliserade mot Ara h 8 och inte mot Ara h 1, h 2 eller h 3 angav jordnötssymtom i lägre utsträckning och angav betydligt lindrigare symtom. Resultaten tydde alltså på att antikroppar mot Ara h 8 kunde ses som indikation på att symtom från jordnöt endast blev lindriga eller att inga symtom uppträdde alls.

Det fjärde delarbetet i avhandlingen är en fallbeskrivning av en 15-årig pojke som fick en anafylaxi efter en jordnötsprovokation på Sachsska barnsjukhuset, trots låga nivåer av jordnötskomponenterna Ara h 1, h 2 och h 3. Analys av allergiantikroppar visade höga nivåer mot jordnötskomponenten Ara h 6 som är mycket lik Ara h 2 i sin proteinstruktur. IgE mot Ara h 6 finns sällan i blodet utan samtidig förekomst av allergiantikroppar mot Ara h 2. Så var det dock inte i detta sällsynta fall.

I studie 5 ville vi testa hypotesen att vid sensibilisering mot jordnöt, men bara förekomst av Ara h 8 antikroppar, uppstår inte allvarliga reaktioner mot jordnöt. Vi gjorde detta genom att utföra jordnötsprovokationer på barn som ”bara var Ara h 8”-sensibiliserade. De fick äta jordnötter under kontrollerade former på Sachsska barnsjukhuset. Nästan alla dessa barn tålde jordnöt eller fick snabbt övergående klåda i munnen. En pojke som reagerat mot jordnöt tidigare i livet fick en kraftig allergisk reaktion vid jordnötsprovokationen i vår studie. En specialanalys av hans IgE-antikroppar, så kallad immunoblotting, visade att nästan allt jordnöts-IgE var riktat mot ett hittills okänt jordnötsprotein. Troligtvis var det IgE mot detta ännu inte karaktäriserade jordnötsprotein som gav upphov till reaktionen.

Sammanfattningsvis talar resultaten för att sensibilisering mot luftburna allergen under barndomsåren är en dynamisk process och att björkpollensensibilisering dominerar vid åtta års ålder. Korsreaktioner mot den björkrelaterade jordnötskomponenten Ara h 8 är sannolikt inte kopplade till svåra allergiska reaktioner vid jordnötsexponering, men det är viktigt att vara vaksam för att hittills okända allergiframkallande jordnötskomponenter i mycket sällsynta fall kan framkalla sensibilisering och reaktioner. Varningstecken är en sjukhistoria med uppgifter om tidigare allvarlig reaktion mot jordnöt eller stigande IgE mot jordnötsextrakt utan motsvarande stegring av IgE mot kända jordnöts-komponenter.

Ca 1/3 av de barn i Sverige som har jordnötsantikroppar i blodet har bara typen Ara h 8. I princip samtliga barn med jordnötsantikroppar i blodet har hittills fått rådet att helt undvika jordnötter av rädsla för allvarliga reaktioner. Den ökade kunskapen kommer underlätta diagnostiken av jordnötsallergi och minska lidandet hos många jordnötssensibiliserade barn som inte längre behöver oroa sig för allvarliga jordnötsreaktioner.

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