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Eczema in young children

- aspects of clinical investigation and treatment

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"Freedom is just another word for nothing left to lose..."

*To my wonderful family
- with all my love*

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Abstract

Background: Eczema affects at least 20 % of children worldwide, and 1/3 of them also have food allergy. In most children, the food allergy is temporary. Improved clinical management and better understanding of etiological mechanisms underlying the tolerance development are target issues in paediatric research.

Study design: The thesis is based on two study groups. The first is a large group of children with suspected allergy investigated with skin prick test in a cross-sectional study. The second group is a cohort of infants with eczema and/or suspected food allergy before 2 years of age, investigated prospectively with follow-up to 4.5 years of age.

Safety of skin prick test (SPT): 5908 children with a mean age of 6.4 years (range: 1 month – 18 years) were investigated with SPT. Seven children, *i.e.* 0.12%, displayed a generalized allergic reaction (GAR), necessitating pharmacological treatment. Seven children showed a vasovagal reaction (VVR). Risk factors for GAR were age < 1 year (RR 6.28) and eczema (RR 16.98). The risk for VVR was highest among female adolescents, and children investigated with multiple skin pricks.

The effect of skin care and food elimination on eczema in infants: 123 children, 52 girls and 71 boys, with a mean age of 8.4 months (range: 1-24 months) were recruited due to eczema and/or suspected food allergy. For diagnosis of eczema, the Hanifin and Rajka criteria were used, and for scoring of eczema severity SCORAD. The infants were investigated twice with an interval of 6 weeks. 62% showed positive SPTs. The SCORAD was higher among the sensitized children before treatment compared to not sensitized children. After treatment, *i.e.* skin care for all and elimination diet for sensitized children, there was no difference regarding eczema severity. Both SPT-positive and SPT-negative children decreased their SCORAD values significantly after treatment. A SPT-negative subgroup, with circulating specific IgE to milk/egg, was only treated with skin care, but these children improved their eczema to the same extent as those also treated with an elimination diet.

Serum and salivary antibodies and achievement of tolerance Analyses were performed regarding: serum levels of total and egg- and milk-specific IgE antibodies, IgG₁ and IgG₄ antibodies to β -lactoglobulin (BLG) and ovalbumin

(OVA); and salivary levels of total IgA, total SIgA and salivary IgA antibodies to OVA and BLG. Samples were drawn at inclusion, after 6 weeks of intervention (skin care, elimination diet), and at 4.5 years of age. Children sensitized to egg and/or milk who had developed tolerance at 4 ½ years of age had higher levels of IgG₄ antibodies to OVA and BLG and also higher IgG₄/IgE ratios on inclusion in the study, than those who remained non-tolerant. The highest IgG₄/IgE ratios were found in children with circulating IgE antibodies to egg and/or milk but negative SPT on inclusion. The six-week treatment period did not significantly affect the levels of serum and salivary antibodies.

Recipes and outcomes of open and double-blinded food challenges in children: After development of recipes for open and blinded challenge with cow's milk and egg, 52 challenges were performed in 39 children. 4 children, challenged blindly, had a positive outcome of the challenge.

General conclusions: The risk for generalized allergic reactions at SPT is low among children and teenagers, but allergic reactions do occur, and low age and eczema are risk factors. Vasovagal reactions occur as often as allergic reactions. Skin care gives significant improvement of eczema severity. Elimination diet may not be needed in infants with sensitization to milk and/or egg, provided that the skin care is adequate.

High ratios of serum IgG₄/IgE antibodies to food allergens may be associated with faster achievement of clinical tolerance, and may support the concept of benefit from continuing allergen exposure in sensitized children.

Recipes for masking of cow's milk and egg in open or blinded food challenges may help to accomplish challenges in young children, often suspicious to unfamiliar tastes or textures.

Sammanfattning

Bakgrund: Eksem förekommer hos 10-20% av barn i hela världen. En tredjedel av barnen med eksem har födoämnesallergi. Hos de flesta växer födoämnesallergin bort innan skolåldern. Förbättrat kliniskt omhändertagande och bättre förståelse av hur klinisk tolerans uppkommer är viktiga mål för forskning inom barnmedicin.

Studieupplägg: Denna doktorsavhandling baseras på studier av två grupper av barn. Den första är en stor grupp med misstänkt allergi som undersökts med pricktest vid ett tillfälle. Den andra gruppen består av små barn med eksem och misstänkt födoämnesallergi. Barnen påbörjade studien innan två års ålder och har sedan följts över tid till fyra och ett halvt års ålder.

Säkerhet vid pricktest: 5908 barn med en medelålder på 6 år och 5 månader, undersöktes med pricktest (SPT). Sju barn (0,12 %) reagerade med generaliserad allergisk reaktion (GAR), och behövde antiallergisk medicinering. Sju barn reagerade vasovagalt (VVR) med svimning eller ”nära-svimning”. Riskfaktorer för GAR var ålder <1 år (RR 6,28) och aktivt eksem (RR 16,98). Risken för VVR var högst hos tonårsflickor och barn/ungdomar undersökta med många allergen (många prickar) samtidigt, oavsett om de var positiva eller inte.

Effekt av lokalbehandling och födoämneselimination hos spädbarn med eksem: 123 barn, 52 flickor och 71 pojkar deltog i studien. Åldern varierade mellan 1-24 månader, med en medelålder på 8,4 månader vid studiens början. Kraven för att få delta var eksem och/eller misstänkt födoämnesallergi. Diagnos av eksem gjordes med stöd av Hanifin och Rajkas kriterier. Eksemgrad bedömdes med instrumentet SCORAD. Barnen bedömdes vid två tillfällen med ca sex veckors mellanrum. 62 % av barnen hade positiv pricktest för födoämnen. SCORAD-värdena i gruppen med positiv pricktest var högre än i gruppen med negativ pricktest, barnen som var födoämnessensibiliserade hade alltså svårare eksem.

Efter sex veckors behandling; födoämneselimination+ lokalbehandling hos SPT-positiva barn; endast lokalbehandling hos SPT-negativa barn; var det ingen skillnad i eksemens svårighetsgrad mellan de två grupperna. Både födoämnessensibiliserade och icke födoämnessensibiliserade förbättrades signifikant av behandling. En grupp med negativ pricktest, men med påvisade antikroppar mot födoämnen i blodet (analyserade först i efterhand), som

behandlades enbart med lokalbehandling förbättrade sina eksem lika mycket som de barn som också ställts på eliminationskost.

Antikroppar i blod och saliv i relation till toleransutveckling: Serumnivåer av total- samt ägg- och mjölkspecifika antikroppar av IgE, IgG₁ och IgG₄ analyserades. I saliv analyserades totalnivåer av sekretoriskt IgA samt specifikt IgA mot mjölk och ägg. Prover togs vid studiens början, efter sex veckor samt vid 4,5 års ålder. Barn som var sensibiliserade mot mjölk och/eller ägg, men som tålde dessa födoämnen vid 4,5 års ålder hade högre IgG₄ nivåer och högre IgG₄/IgE-kvot vid studiens början, än de barn som ej uppnått tolerans. De högsta IgG₄/IgE-kvoterna sågs hos barnen med negativt pricktest men positivt specifikt IgE i blod. Under den första korta observationsperioden på sex veckor sågs ingen påverkan på barnens antikropps-nivåer.

Recept/metodutvecklande och resultat av öppna och dubbel-blinda placebo-kontrollerade födoämnesprovokationer: Efter recept och metodutveckling för födoämnesprovokationer med mjölk och ägg, utfördes 52 provokationer på 39 barn. Fyra barn, alla provocerade blint, reagerade på provokationen.

Generella slutsatser: Risken för generaliserade allergiska reaktioner vid pricktest är liten hos barn och tonåringar, men den finns. Riskfaktorer är låg ålder och aktivt eksem. Vasovagala reaktioner är lika vanliga som generaliserade allergiska reaktioner.

Lokalbehandling/smörjning ger signifikant förbättring av eksem. Elimination av födoämnen kanske inte är nödvändigt hos eksembarn med sensibilisering för mjölk och ägg under förutsättning att hudvården sköts noga.

Höga IgG₄/IgE-kvoter av specifika antikroppar mot födoämnen kan vara associerat med snabbare toleransutveckling, och kan stödja idén med fortsatt allergenexponering hos födoämnessensibiliserade barn.

Recept på beredningar som väl maskerar komjölk och ägg, vid öppna och blindade födoämnesprovokationer, är en god hjälp vid provokationer av små barn som ofta är misstänksamma mot nya smaker och konsistenser av mat.

List of original papers

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I** Adverse reactions to skin prick tests in children: prevalence and possible risk factors.
Norrman G and Fälth-Magnusson K.
Submitted.
- II** Significant improvement of eczema with skin care and food elimination in small children.
Norrman G, Tomičić S, Fagerås Böttcher M, Oldaeus G, Strömberg L, Fälth-Magnusson K.
Acta Paed 2005; 94:1384-88.
- III** High ratios of IgG₄/IgE antibodies to food allergens are associated with faster achievement of tolerance in food sensitized infants with eczema.
Tomičić S, **Norrman G**, Fälth-Magnusson K, Jenmalm MC, Devenney I, Fagerås Böttcher M.
Submitted.
- IV** A new model for low-dose food challenge in children with allergy to milk and egg.
Devenney I, **Norrman G**, Oldaeus G, Strömberg L, Fälth-Magnusson K.
Acta Paed 2006; 95:1133-9.

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Abbreviations

AE	Atopic Eczema
AEDS	Atopic Eczema/Dermatitis Syndrome
APT	Atopy Patch Test
AU	Arbitrary Units
BLG	β -lactoglobulin
BSA	Bovine Serum Albumin
CD	Cluster of Differentiation
cNOS	constitutive Nitric Oxide Synthase
CV	Coefficient of Variation
DBPCFC	Double-Blind Placebo-Controlled Food Challenge
EACCI	European Academy of Allergology and Clinical Immunology
GAR	Generalized Allergic Reaction
HSA	Human Serum Albumin
ICT	Intracutaneous skin Tests
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	InterLeukin
iNOS	inducible Nitric Oxide Synthase
ISAAC	International Study of Asthma and Allergies in Childhood
kU/l	kiloUnits per liter
LAR	Local Allergic Reaction
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OAS	Oral Allergy Syndrome

OD	Optical Density
OVA	Ovalbumin
PBS	Phosphate-Buffered Saline
PUVA	photo chemotherapy with Psoralen and Ultraviolet A
RAST	Radioallergosorbent Test
SAFT	Skin Application Food Test
SCORAD	Severity Scoring of Atopic Dermatitis
SIgA	Secretory Immunoglobulin A
SOTI	Specific Oral Tolerance Induction
SPT	Skin Prick Test
Th	T-helper cell
VVR	VasoVagal Reaction
WAO	World Allergy Organization

Introduction

A historical view

Allergic diseases were described already in the days of Hippocrates, who used the word eczema (*Greek ek: out; zeo: boil*) to describe skin rashes. The term allergy (*Greek allos: other; ergon: reaction*) was introduced by the Austrian paediatrician Clemens von Pirquet in 1906 [1].

The concept of atopy was first presented by AF Coca and RA Cooke in 1923 [2], when they described hypersensitivity diseases characterized by an immediate-type wheal reaction, allergic manifestations such as asthma, eczema and hay fever, and circulating reagins. Eczema connected to food allergy was described by Talbot [3] when he found that patients with eczema significantly improved after elimination of suspected foods. MH Loveless was the first to use blinded placebo-controlled food challenges to determine whether a patient was allergic to a suspected food [4].

Nomenclature today

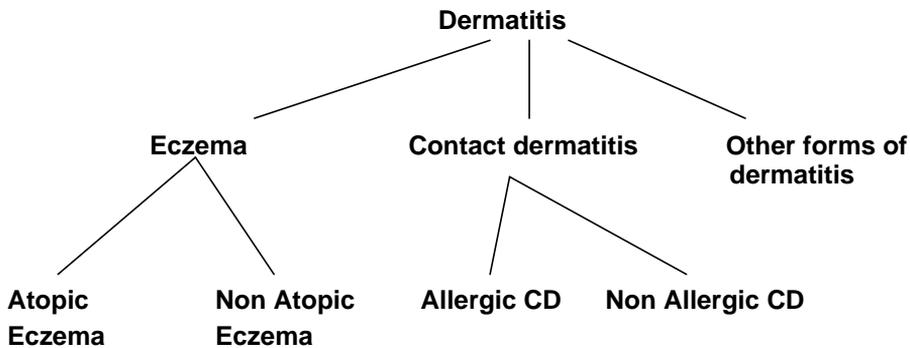
The different terms cited above have been used alternatively over the years. In 2003 the European Academy for Allergy and Clinical Immunology (EAACI) proposed a revised nomenclature for allergic diseases [5] which was accepted by the World Allergy Organization (WAO). The nomenclature is based on the mechanisms initiating the reaction, and could be used independently of target organ and age of the patient.

Allergy is a hypersensitive reaction initiated by specific immunological mechanisms. It can be antibody- or cell mediated. In patients with allergic symptoms from the gastrointestinal tract or the airways, most have an **IgE – mediated allergy**.

Atopy is a personal and/or familial tendency to become sensitized and produce IgE-antibodies when exposed to allergens. It is a clinical definition of an IgE-antibody high-responder.

An **allergen** is an environmental antigen (usually a protein) causing allergic disease.

The term *eczema* has replaced the former AEDS (Atopic Eczema/ Dermatitis Syndrome) [6]. Eczema is thus located below Dermatitis in the umbrella (see below)



Food allergy is caused by immunological reactions to food allergens. If IgE is involved the term *IgE-mediated food allergy* is appropriate.

Anaphylaxis is a severe, life threatening generalized or systemic hypersensitivity reaction. Allergic anaphylaxis is caused by immunological mechanisms.

Heredity and the “atopic march”

Allergy (IgE-mediated) is a hereditary disorder, the disposition to form allergen specific IgE is probably polygenetic [7]. The early environment of the child also contributes a great deal to how the disease will develop in an individual child. Some of the factors proposed to affect allergy development are exposure to tobacco smoke, infections, damp houses, life style matters, and of course allergens.

The natural history of atopic manifestations is often referred to as the “atopic march”. An atopic individual usually presents with atopic eczema and food allergy during infancy. It can start as early as in the first months of life, and the process has as a rule started before two years of age. The children often outgrow their food allergy, but it can be a variable process. Eighty percent of children outgrow their milk- and egg allergy before school start [8, 9], whereas allergies to peanuts and tree nuts often remain [9]. The next step in the “atopic march” is sensitization to aeroallergens, and further development of allergic rhinitis and asthma. Eighty percent of children with atopic eczema develop sensitization to

aeroallergens [10] but not all of them develop any clinical symptoms. The conformity of the “atopic march” has been questioned, especially regarding respiratory symptoms in early age. Wheezing can be the first symptom of an atopic disease, but many of these infants do not have an increased risk of asthma later in life [11].

Prevalence of allergy

In spite of the fact that the terms for allergic conditions have been known for a long time, it seems to have been rare diseases in the 19th century. The London physician John Bostock needed 10 years to find approximately 30 patients with seasonal allergic rhinitis [12]. During the last decades the prevalence of allergic diseases has increased substantially [13] according to reports from developed countries. The prevalence of eczema has increased two- to threefold during the last three decades [10, 14] reaching 10-20% in children. The prevalence of food allergy in childhood is 6-8%, with a peak at one year of age [9, 15, 16]. A world wide study (ISAAC) has been conducted regarding changes in the prevalence of asthma, allergic rhinoconjunctivitis and eczema [13, 17]. When comparing results from phase I (mostly 1994-1995) with phase III (mostly 2002-2003) there is an increase in most of the participating centers in the age group 6-7 years. It can thus be suspected from this report [17] that the increasing prevalence in developed countries is reaching a plateau, since there are decreases reported in the older age group 13-14 years in countries with high prevalence.

Immunology

The main task of the immune system is to recognize self and non-self, and to be able to eliminate foreign invaders. The immune responses are divided into:

Innate immunity: which provides the first line of defence and is mediated by granulocytes, macrophages, dendritic cells and natural killer cells [18, 19].

Adaptive immunity: which is mediated by lymphocytes, and is antigen specific [18, 19].

These systems cooperate both against infections and in other immune responses [20]. The adaptive system is activated by antigen exposure. The cells involved in the adaptive response are B-lymphocytes which differentiate into antibody-producing plasma cells and T-lymphocytes which includes T-helper cells and T-cytotoxic cells. The cells of the immune system communicate with stimulatory and inhibitory cytokines.

Allergic disease is the result of a reaction to harmless antigens (allergens) that should normally be tolerated.

Allergic immune mechanisms

IgE antibodies to both dietary and inhaled allergens appear in both atopic and non-atopic subjects, but the response is down-regulated with age in the non-atopic subjects [21].

The sensitization process is initiated by the induction of allergen specific Th2-like cells that produce IL-4, which induce IgE switch and promote allergen specific IgE-antibody production from B-cells [22]. The IgE antibodies attach to mast cells and basophils via the high affinity FcεRI –receptors [22]. At re-exposure to the allergen there will be a cross-linking of the allergen specific IgE-antibodies on mast cells resulting in release of inflammatory mediators such as histamine, tryptase and chymase, within the range of a few minutes. The mast cells also synthesize mediators that induce a more sustained inflammation in the organs affected.

IgG antibodies to allergens are produced in both atopic and non-atopic children, with a peak in early childhood and decline by eight years of age [23, 24]. The levels of allergen specific antibodies of the IgG-isotype are often higher than the IgE-levels in the non-atopic group [25]. Particularly the subclass IgG₄ is associated with allergy and atopic sensitization [23]. There are similar but diverging mechanisms regulating production of IgE and IgG₄. Th2 derived IL-4 induces production of both [26], but IL-10 inhibits IgE-production and up-regulates the secretion of IgG₄ [27]. It has been shown that a high IgG₄/IgE ratio for airborne allergens may favour immunological tolerance development [28].

In humans the predominant antibody on the mucosa is secretory IgA, produced locally and situated along the whole mucosal outlining. Specific IgA prevents adherence of allergens and thus penetration and sensitization (at least theoretically). Low levels of IgA and transient IgA deficiency have been associated with an increased risk of allergy [29, 30]. Development of clinical allergy seems to be associated with high levels of total and allergen-specific IgA in serum, but low SIgA. High levels of SIgA seem to protect sensitized children from developing clinical allergy in the first 2 years of life [31]. The levels of secretory IgA increase with age [31], and this increase has been suggested to occur more slowly in allergic children [32].

Methods of investigation

It is recommended that all individuals with severe, persistent or recurrent possible “allergic symptoms” should be tested for specific allergy [15]. This can be accomplished by skin tests for allergy, by in vitro allergy tests or by challenge procedures.

Skin tests for allergy

It was first shown by Prausnitz and Küstner [33] that sensitization can be demonstrated on the skin. Skin tests for allergy can be performed as intracutaneous (intradermal) skin tests (ICT) or skin prick (puncture) test (SPT). Earlier a skin scratch test was used, but it is no longer recommended because of the low specificity [34]. The skin prick test is considered the test of choice.

Skin prick test: The SPT can be performed on the volar surface of the forearm or on the back. The test should not be performed on a location with eczema or obvious dermatographism. If a limited number of tests are to be applied, the volar surface of the arm is most convenient [35]. The different tests should be placed 3 cm apart and not closer than 5 cm from the wrist and 3 cm from the elbow. The lancet used nowadays is often one designed for this purpose with 1 mm tip and shoulders preventing further penetration. The lancet should be pressed at 90 degrees to the skin surface through a drop of test solution. Antihistamine drugs should be discontinued > 72 hours before skin test. Allergens used should be strictly standardized [34, 35], and contain active allergens in amounts resembling natural sources of the respective allergen. Some allergens, *e.g.* food allergens are not available as extracts, or not considered reliable due to protein degradation. For those one can use the prick-prick method, when one pricks with the lancet into for example a fruit and then pricks the skin [35]. A negative control solution or a blank lancet should be used in parallel with allergen tests to rule out for example dermatographic reaction to test devices only. A positive control, *e.g.* histamine, is used to document normal reactivity of the skin. The positive control is also used for comparison when recording positive tests. The size of the wheal is documented 15 minutes after test, and is measured/recorded as the mean diameter of the longest and the midpoint orthogonal diameters. Wheals should be at least 3 mm in diameter and greater than the negative control to be considered positive [15]. There is a circadian rhythm of the size of skin reactions with maximum wheal size during the night. In a non-atopic population the reaction to histamine varies with age, but in children with manifest atopic allergy the skin reactivity is similar from 1 year of age until puberty [36].

Skin application food test (SAFT) is a patch test applied for only a few minutes, which has been suggested to be useful in detecting early phase type 1 immediate hypersensitivity reactions [37]. It is regarded as a child friendly test since no needle is used. This test was compared to SPT and APT (atopy patch test) in diagnosing egg allergy in a study by Hansen et al [38].

The **atopy patch test (APT)** is another test for atopy/allergy which can be used for investigation of atopic eczema, but this test needs further standardization before it can be used as a clinical standard test [39-42].

Neither SAFT nor APT seems to increase the diagnostic accuracy in children with atopic eczema [38].

In vitro allergy tests

Total IgE: The normal value for total IgE increases gradually up to prepuberty when it reaches adult levels [43, 44]. Earlier total IgE has been used as a marker for allergy, but normal total IgE levels do not rule out specific allergy [15] and high levels of total IgE are not always associated with clinical symptoms.

Specific IgE: Tests for specific IgE should be conducted with a validated method [45, 46]. Qualitative in vitro assays (*e.g.* RAST) provide suggestive evidence of IgE-mediated food allergy, but these assays are giving way to the quantitative tests, which have shown to be more predictive of symptomatic IgE-mediated allergy [47]. The UniCap^R system is an in vitro immunoassay, which measures the concentration of circulating allergen-specific IgE in human serum or plasma. The assay is calibrated against the World Health Organization standard for IgE [48]. The levels of specific IgE is reported in values of concentration, but can also be translated into classes as shown below.

IgE-class	IgE kU/l
0	<0.35
1	0.35-0.7
2	0.7-3.5
3	3.5-17.5
4	>17.5

Specific IgE in sera measures circulating specific IgE, but in some cases IgE may be produced locally and only found in the shock organ, resulting in allergic reactions despite low levels of specific IgE in sera [49].

Eczema

Etiology

Atopic eczema (AE), (see umbrella, page 10) is a genetically complex disease, with a strong maternal influence. Hereditary factors are stronger for AE than for asthma or allergic rhinitis, suggesting specific genes connected to AE [10].

Skin pathology

Both clinically affected and unaffected skin in patients with AE is abnormal. The unaffected skin reveals a mild hyperkeratosis and a sparse perivascular T-cell infiltration [10]. In acute eczematous lesions a marked intracellular oedema (spongiosis) can be seen. Antigen-presenting cells have IgE-molecules attached, and in dermis there is a striking infiltration of CD4-activated T-cells. In chronic lesions one can see an acanthotic epidermis, with elongated rete ridges and parakeratosis, but not so much of the spongiosis found in acute lesions. There are also an increased number of IgE-bearing Langerhans cells, mastcells and inflammatory dendritic cells in the epidermis. Macrophages dominate the dermal mononuclear cell infiltrate [10, 50].

Clinical diagnosis

Atopic eczema is a criteria-based diagnosis, and many sets of criteria have been proposed, such as the Hanifin and Rajka criteria (Table 1), the Schulz - Larsen criteria, the Danish Allergy Research Centre criteria, the UK Working party's criteria and Williams criteria (Table 2). The Hanifin and Rajka criteria are widely used and have a high degree of specificity [51]. The Williams criteria are a simplified version derived from these criteria [52]. According to Leung and Bieber the following essential and associated features can be derived from the described criteria [10], see below.

Essential features:

Pruritus
 Facial and extensor eczema
 Flexural eczema in adults
 Chronic or relapsing dermatitis

Frequently associated features:

Personal or familial history of atopic disease.
 Xerosis
 Cutaneous infections
 Non-specific dermatitis of hand and feet.
 High IgE-levels
 Early age of onset
 Positive immediate type reaction

Basic features	Minor features
Pruritus	Xerosis
Typical morphology and distribution – facial and extensor involvement in infants	Immediate (type1) skin test reactivity
Chronic or chronically relapsing eczema	Elevated levels of serum IgE
Personal or family history of atopy	Early age of onset
	Tendency towards cutaneous infection (Herpes simplex; Staph Aureus)
	Cheilitis: chronic desquamation of upper or both lips and even the perioral areas
	Recurrent conjunctivitis
	Dennie-Morgan infraorbital fold
	Orbital darkening
	Facial erythema or pallor
	Itch when sweating
	Intolerance to wool and lipid solvents
	Food intolerance
	Course influenced by environmental or emotional factors

Table 1: Hanifin and Rajka criteria of atopic eczema, modified for children. For diagnosis 3 of 4 basic and 3 or more minor features are needed.

Child must have had itchy skin condition in past 12 months
+ three or more of
<ul style="list-style-type: none"> • History of involvement of skin creases
<ul style="list-style-type: none"> • Personal history of asthma or hay fever Or in 1st degree relative if aged < 4 years
<ul style="list-style-type: none"> • History of generally dry skin in past year
<ul style="list-style-type: none"> • Visible flexural dermatitis Not used in children aged < 4 years
<ul style="list-style-type: none"> • Onset below age 2

Table 2: Williams criteria

The feature of atopic eczema varies with age:

Infants (<2 years):

Acute or sub acute course
Intensely pruritic, erythematous papules
Excoriations
Exudative areas with crusts
Involves face (cheeks and chin), scalp, trunk and extensor surface of the extremities

Preschool and schoolchildren (> 2 years):

Chronic course
Lichenification, papules and excoriation
Location: Neck, wrists, ankles and flexural folds of the extremities

Triggers

The most investigated, and described triggers, worsen or induce the AE by immunological mechanisms.

Food allergens induce skin rashes in 40% of children with moderate to severe eczema [8]. Food allergens might induce dermatitis and contribute to the severity of skin disease. T-cells specific to food allergens have been cloned from skin lesions of patients with AE [10].

Aeroallergens: Bronchial or intranasal inhalation of aeroallergens has been shown to induce pruritus and skin lesions in sensitized patients with AE. Also epicutaneous application of aeroallergens on unaffected skin in patients with AE can induce these reactions [10].

Autoallergens: The allergic inflammation in the skin can be maintained by endogenous antigens [10].

Staphylococcus aureus: Secreting of superantigens stimulates T-cells and macrophages.

Other triggers to be mentioned are irritants such as soaps and detergents, and emotional stress.

Treatment

The treatment for AE is predominantly local, and should aim to relieve the xerosis, the inflammation of the skin, the itch and any secondary skin infections.

Skin care: Softening skin care products with carbamide and/or lactic acid should be applied at least twice daily. These products help to hydrate the skin, prevent evaporation and allow autorepair through reconstruction of new lipids. Soap should only be used when needed, and should have minimum defatting activity and a neutral pH [10].

Anti-inflammatory treatment: Topical glucocorticoids are the drug of choice. A medium- to high potent glucocorticoid is used daily to achieve control of an acute exacerbation. Once control of the eczematous areas is achieved, long term control can be maintained by a twice-weekly application on locations prone to develop eczema. Side effects of local glucocorticoids are dependent of potency and length of use, and high-potency agents should therefore be used only shorter periods and never in the face or intertriginous areas [10].

Tacrolimus and pimecrolimus (calcineurin inhibitors) are anti-inflammatory agents that inhibit production of Th1 and Th2 cytokines and mediator release

from mast cells. They are proposed when the effect of topical glucocorticoids is insufficient, in patients with grave steroid phobia, and when more effective treatment is needed in the face. These drugs, as being relatively new, require careful monitoring if used long-term [10].

Anti-infectious management: Since patients with AE are more frequently colonized with staphylococcus aureus, anti-staphylococcal antibiotics can be very helpful in poorly-controlled patients. Both topical and systemic preparations can be used, depending on severity.

Recurrent viral infections are also a problem, such as warts and mollusca contagiosa. Although very rare a widespread infection with herpes simplex (eczema herpeticum) can be life-threatening and antiviral treatment is crucial. Fungal infections are more common in patients with atopic eczema than in non-atopic controls, and topical antifungal treatment may be needed [10, 53].

Itch-management: Control of the itch is crucial because scratching maintains and aggravates the eczema. Topical anti-inflammatory agents and skin care is first-hand choice. Sometimes oral antihistamines can be useful. Avoidance of dust, woollen fibers, water and soap tempers itch, whereas stress and sweating may worsen it [10, 53].

Phototherapy: Natural sunlight is beneficial to most patients with AE, but if it is combined with hot climate and high humidity, the eczema could worsen. Combined or separated ultraviolet A and B phototherapy is often a useful adjunct in treatment of AE. Photo chemotherapy with psoralen and ultraviolet A beams (PUVA) should be restricted to patients with severe, widespread AE [10].

Food allergy

Food allergy is defined as an adverse immune response to food proteins. It affects 6% of young (<3 years) children [47, 54]. Food induced allergy reactions are responsible for symptoms from a variety of organ systems, such as the skin, the gastrointestinal tract and the respiratory tract. Food is also the most common cause of outpatient anaphylaxis [55].

Pathogenesis

The sensitization to food allergens may be divided into two different mechanisms.

Class 1 food allergy: A breach in the development of oral tolerance in the gastrointestinal tract while the food allergens are being ingested.

Class 2 food allergy: Sensitization to food allergens apart from the gastrointestinal tract, instead sensitization occurs when exposed in the respiratory tract (*e.g.* cross reaction to pollen, pollen-food related syndrome, oral allergy syndrome (OAS)) [54].

Class 1 allergens, such as egg and peanut, may also evade oral tolerance by initial sensitizing exposure through the skin [56]. Any abrogation of the complex gut barrier might promote food allergy [54].

The precise mechanism behind oral tolerance is not fully understood. However high-dose tolerance involves deletion of effector T-cells, whereas low-dose tolerance is mediated by activation of suppressive regulatory T-cells [57]. Why some individuals fail to induce oral tolerance is still obscure, but the importance of the normal gut flora has been suggested as one contributing factor [58].

Food allergens

The list of causal food allergens is relatively short, the most common are cow's milk, egg, peanut, wheat, soy, tree nuts, fish and shellfish [47, 54, 59]. The allergy to food allergens in early childhood (egg, milk, soy and wheat) usually resolves before school age (approximately 80%). Allergy to peanut, tree nuts, and seafood usually become permanent [9, 47, 54], although there are exceptions. Peanut allergy resolves before school age in 20 %, but recurrence occurs in some cases [60, 61].

Class 1 allergens are water soluble glycoproteins 10 - 70 kD in size, stable to heat, acid and proteases, for example milk (caseins), peanut (vicillins), egg (ovomuroid) and non-specific lipid transfer proteins found in apple.

Class 2 allergens are though often changed by heat, and heating of food might reduce or enhance allergenicity, for example Mal d 1 in apple or Dau c 1 in carrot [54].

Diagnosis

The first and most important step is to reveal a thorough history from the family, determining possible causal foods, quantity, time course and ancillary factors such as exercise.

The second step is to determine specific IgE, by SPT or serum test. Increasing wheal size and increasing IgE concentration increases the likelihood of a clinical reaction. Negative SPT has a negative predictive accuracy of >95%. A negative food-specific IgE might be associated with clinical reaction in 10-25% [16], so if the history points at food allergy a negative SPT and/or a negative food challenge is necessary to confirm absence of food allergy [47, 54, 59]. The double-blind placebo-controlled oral food challenge is the gold standard for the diagnosis of food allergies.

Treatment

The primary therapy is to avoid causal foods. In order to avoid nutritional deficiencies a dietician is an indispensable member of the treatment team. Elimination of basic food items *e.g.* milk and all dairy products, imposes a risk of inadequate nutritional supply, unless an adequate substitute is provided [62]. Since most childhood food allergies resolve, it is important to repeat evaluations regularly (every 1-2 years).

Pharmacological treatment: Antihistamines can relieve OAS and IgE-mediated skin symptoms. Systemic corticosteroids are effective, but long term treatment is unacceptable because of side effects.

Immunotherapy: Injections of anti-IgE antibodies to patients with peanut allergy has shown quite promising results [63]. Standard immunotherapy for birch pollen might improve OAS, but confirmation studies are needed [64]. Specific oral tolerance induction (SOTI) is a new model reviewed by Niggeman [65], with promising results. This method is performed with small doses of the offending food administered daily in slowly increasing doses until amounts resembling ordinary intake is reached. So far a regular intake of the food seems to be needed to maintain tolerance, but a much appreciated goal could be better tolerance at accidental ingestions [66].

Prevention: Although constantly debated, many studies suggest a beneficial effect of exclusive breastfeeding for the first 3-6 months in high risk children, and if breastfeeding is not possible, supplementary formulas should be hypoallergenic [67-69]. Manipulation of the mothers diet during pregnancy and lactation, or restriction of allergenic foods in the infants diet has not been effective in preventing allergy development [56, 67, 70-72].

Oral food challenge

The most practical initial approach to screen for food allergy is an open or single blind food challenge directed by SPT [73]. Ideally, all children should undergo double-blinded placebo-controlled food challenge (DBPCFC) which is the only appropriate and reliable method for evaluating and confirming a suspected adverse food reaction, and thus referred to as “the gold standard” [74-76].

Recently general advice for standardization of the method has been suggested by a working group in EAACI [77]. A protocol for low-dose food challenge, aiming at defining threshold levels has been suggested [78]. The models do not fully meet the demands for food challenge in childhood, where masking of the smell, flavour and texture is essential. Further they are not suitable for children outgrowing their food allergy, because it is important to show parents that their child can eat amounts resembling a normal portion of the food without getting an allergic reaction, and because administration in children demands greater detail care and often fantasy.

Food challenges in children should always be performed by an experienced paediatrician [79].

The algorithms presented for when to perform food challenges based on measurements of specific IgE and SPT outcome [80], should be used with great caution in infants < 2 years of age.

When to perform food challenges

The oral food challenges should be performed [77]:

In children with a history of adverse reaction to food:

- to establish or exclude the diagnosis of food allergy
- to determine the threshold value or degree of sensitivity
- to assess tolerance when outgrowing the food allergy
- for scientific reasons in clinical trials

In children without specific history of adverse reaction to food:

- if any chronic symptom is suspected by the patient or the physician to be food related
- if the child is on an improper elimination diet without history of adverse food reaction
- if sensitization to food is diagnosed and tolerance is not known, *e.g.* sensitization to cross-reactive foods

The oral food challenge should not be performed [77]:

- in children with a clear history of anaphylaxis or severe systemic reaction to a specific food
- in children with ongoing disease (*e.g.* acute infection, seasonal asthma)
- in children with unstable chronic atopic disease
- in children on treatment with oral antihistamines or oral steroids, which might mask, delay or prevent the evaluation of reaction

Performance

- clinical monitoring should be standardized [81]
- suspected foods should be eliminated 7-14 days before challenge
- natural foods should be offered in the way the patient would normally eat it [74, 81, 82]
- the start dose should be half the minimum quantity estimated by the patient to have produced the symptoms [82]
- the top dose should be a normal amount of a serving of the food, adjusted for age [77]
- the dose can be doubled or raised in a logarithmic mean, with intervals 15-30 minutes [77]
- the concentration of the suspected agent hidden in the food should be as high as possible without being detectable [83]
- the placebo should be identical in flavour, colour and consistency to the active substance [82, 83]

Outcome

A challenge is considered positive when objective symptoms occur within two hours of the oral challenge [84-86]. Clinical reactions after 2 hours are defined as late reactions [74].

Even at DBPCFC false positive (0.7%) and false negative results (3.2%) do occur [16].

Open challenges are sufficient in defining non-reactors but give higher percent of false positive results [82]. In young children (< 2-3 years of age) the open challenge is usually adequate [87].

After a negative food challenge the parents should be instructed to add the tested food to the diet in small, but increasing amounts for several days [88] and pay attention to any adverse reaction. Once the food is fully tolerated it can be eaten as often as the patient desires, and in usual portions [88].

Risks and precautions

Although there is a low risk of generalized reactions, oral challenges should be performed in a hospital setting with both a nurse and a physician present. Rescue medication should be prepared, and at hand. Intravenous access should be available before initiation of the challenge as a general rule. The child should be observed for at least 2 hours after the last dose. If a severe reaction is suspected in advance, the challenge should only be performed in settings with immediate access to an intensive care unit [77].

Aims of the thesis

The general aim of the research presented in this thesis was to prospectively study the clinical and immunological course of eczema and possible food allergy in young children, and to evaluate safety aspects of a common procedure of allergy investigation.

The specific aim of each paper was to:

- I** Study the safety aspects of skin prick test, delineate the prevalence of adverse reactions and evaluate possible risk factors.

- II** Investigate the prevalence of food sensitization in infants with eczema and evaluate the effect of interventions, *e.g.* food elimination and skin care.

- III** Identify immunological parameters related to tolerance development and assess the effect of elimination diet on serum and salivary antibodies.

- IV** Develop recipes and protocols suitable for young children performing food oral challenges with milk and egg, and test these tools in a cohort of children.

Material and methods

The thesis is based on two study groups. The first was chosen to represent a large group of children with suspected allergy who were investigated with skin prick test in a cross-sectional study (Paper I). The second group was a cohort of infants referred for investigation of eczema and/or suspected food allergy before 2 years of age, with follow-up to 4.5 years of age (Paper II-IV).

Paper I: Safety of the skin prick test.

Subjects

11 paediatric settings participated in this study. The departments included were one private practice (A), the unit for allergy research (B), and the paediatric outpatient clinic (C), all in Linköping, the paediatric outpatient clinic in Hudiksvall (D), the paediatric outpatient clinic in Finspång (E), the paediatric outpatient clinic in Motala (F), one private practice (G), and the paediatric outpatient clinic (J), both in Norrköping, the paediatric outpatient clinic in Jönköping (H), the paediatric outpatient clinic in Mjölby (I), and the paediatric outpatient clinic in Växjö (K) (see map in Fig. 1). All units were introduced and informed about the study during a personal visit by the authors. All children (0-18 years) investigated with SPT during the time period 1999-04-01–2001-10-01 were included, and in total 5908 children were investigated. Further information about the subjects is given in Table 3.

Unit	Number of patients	Sex F/M	Female %	Male %	Age mean (years)	Age median (years)
total	5908	2638/3218	45	55	6.4	6
A	331	148/183	45	55	7.5	7
B	922	421/495	46	54	8.0	7
C	1161	509/637	44	56	5.5	4
D	282	125/151	45	55	5.7	4
E	141	68/71	49	51	8.5	8
F	280	95/180	35	65	7.5	7
G	491	230/260	47	53	5.8	5
H	565	234/317	42	58	6.4	5
I	104	48/56	46	54	7.3	7,5
J	702	321/380	46	54	4.6	3
K	929	439/488	47	53	6.8	6

Table 3: Number of patients, age and sex distributed per unit.



Figure 1: This map displays the geographic locations of the participating units.

Documentation

A form sheet was designed for documentation of SPT date, birth data of the patient, number of positive and negative SPTs, grouped in food, animal, pollen and various allergens. The positive tests were specified by allergen using an abbreviation key. In the form it was also documented if the child had eczema (mild, moderate or severe), signs of ongoing infection, and if any adverse reaction occurred. If an adverse reaction occurred, a special form was filled out, with full identity of the child, information about anamnesis, previous treatment, and the reaction and its treatment with possible diagnosis. The complete patient journal text could be requested if needed.

Allergens

Fresh specimens were used for foods, *i.e.* fruits, nuts, and for other allergens the commercial extracts Soluprick SQ^R (ALK-Abelo, Hørsholm, Denmark) were used. The standard allergens are shown in Table 4. A large group of other defined allergens, listed in Table 5, were occasionally used

ANIMAL	POLLEN	VARIOUS	FOOD
Cat	Birch	Alternaria	Almond
Cow	Mugwort	Cladosporium	Cow's milk
Dog	Timothy	D. farinae	Egg
Horse		D. pteronyssinus	Fish
Rabbit			Hazelnut
			Peanut
			Soy

Table 4: Standard allergens categorized in four groups.

Skin Prick Tests

The SPTs were performed in the same way in all units, as recommended by the European Academy of Allergy and Clinical Immunology (EAACI) [89]. SPT with fresh foods was performed with the prick-prick method, and with commercial extracts with the prick-puncture method. Blank lancets served as negative control and Histamine dihydrochloride 10 mg/ml as positive control. The SPT was considered positive when the mean diameter (half of the sum of the largest diameter and its midpoint perpendicular) of the wheal was at least 3 mm, when read after 15 minutes. The size of the wheal was marked with a filter pen and transferred onto a micro pore tape for measuring.

Animal	Insects	Pollen	Various	Food	
Guinea-pig	Bee	Alder	Latex	Apple	Oatmeal
Hamster	Wasp	English rag weed	Penicillin	Apricot	Orange
Pig		Hazel		BabySemp1 [®]	Paranut
				Banana	Pea (yellow)
				Barley	Peach
				Beef	Pecannut
				Cashewnut	Pistagenut
				Chicken	Potato boiled
				Chick-pea	Potato
				Corn	Profylac [®]
				Cod	Rye
				Egg boiled	Sesame seed
				Green pea	Shrimp
				Haricots verts	Walnut
				Kiwi	Wax bean
				Milk boiled	Wheat
				Nutramigen [®]	

Table 5: Other allergens occasionally used.

Adverse reactions

The adverse reactions were classified into three groups according to reaction type.

Generalized Allergic Reaction (GAR): subjective complaints of apprehension, generalized pruritus or flushing, feeling of asphyxiation, tightness of chest, or dizziness and objective findings of itchy watering eyes, sneezing, nasal congestion, rhinorrhea, urticaria, angioedema, cough, wheeze or tachycardia [90, 91]. In infants excessive crying and vomiting in combination with other signs were also accepted as signs of GAR.

Local Allergic Reactions (LAR): Localized allergic reactions such as pruritus, urticaria or rash solely on the test arm.

Vasovagal Reactions (VVR): syncope, when unconsciousness was reported; near-syncope, when light-headedness was reported; or malaise, if other symptoms were reported, as in the study by Turkeltaub and Gergen [92].

The classification of reactions, listed above, was chosen to facilitate comparisons with previous studies.

Ethics

The study was approved by the Human Research Ethics Committee at the Faculty of Health Sciences, Linköping University, and the Medical Faculty at Uppsala University.

Statistics

To compare the result in this study with previous findings, we used Chi-square test. The risk ratios were calculated with the statistical software Stata. The chi-square test and Fishers exact test were employed to evaluate sex distribution, age and eczema in the group with adverse reactions, Spearman's correlation test to evaluate correlation, and one-way ANOVA test to compare subgroups.

Paper II, III, IV. The eczema/food reaction study:

Patients

Children from 4 paediatric clinics were included in this study (Hudiksvall, Jönköping, Linköping and Norrköping). Inclusion criteria were: age < 2 years and admission note because of eczema and/or suspected food allergy. The children were recruited between June 1999 and September 2001, and they were all referred from primary care physicians.

There were 123 children, 52 girls and 71 boys, mean age 8.4 months (range: 1-24 months). Further description of distribution per unit and clinical data is given in Table 6. In Linköping and Hudiksvall analyses were performed to evaluate if the participating children constituted a representative sample. In Linköping 109 families altogether were invited to participate, 53 accepted. The reasons for declination were: language/communication problems 12; participation in other studies 8; other severe diseases 3; not interested 33. In Hudiksvall 18 families were invited, 12 accepted. The reasons for not participating were: not interested in participating 3; no contact by phone or letter 3.

Three of these paediatric clinics are located in the southeast of Sweden (Jönköping, Linköping and Norrköping), and one in the north of Sweden (Hudiksvall). To assure clinical concordance and good cooperation in the study, the main authors (GN and KFM) visited the units to discuss the study design, before start of the study. During the study, all participating nurses and physicians met once or twice a year to discuss research questions and study progress.

UNIT	NUMBER OF PATIENTS	FEMALE/MALE	FEMALE %	MALE %	AGE MEAN (months)	MEDIAN; MIN;MAX (months)
total	123	52/71	42	58	8.4	7; 1; 24
A	12	3/9	25	75	8.8	5; 4; 20
B	44	21/23	48	52	9.9	8; 3; 24
C	53	22/31	42	58	7.8	6; 1; 23
D	14	6/8	43	57	5.8	6; 2; 19

Table 6: Description of patients and distribution on the four participating units

Eczema assessment

The diagnosis eczema was set, using the Hanifin and Rajka criteria as described previously (page 16). At each clinical visit the eczema was evaluated with the SCORAD (Severity Scoring of Atopic Dermatitis method) [93], which is a standardized method for assessing eczema, taking into account the extent and severity of the eczema as well as the consequences of the skin disorder (degree of pruritus and sleeping disorder). We used the form designed by the inventors translated into Swedish (Fig. 2). The children were judged to have mild (SCORAD<25); moderate (SCORAD 25-50); or severe (SCORAD >50) eczema. Before the start of the study, all nurses and physicians practised scoring on a child with eczema to evaluate interobserver variability. During the study, each child was evaluated by the same investigator, before and after treatment, to reduce the interobserver variability.

Examinations

Visit 1 was made at the time of inclusion. Visit 2 was made after approximately 6 weeks. Then the group with positive SPT came once a year to the clinic, and the group with negative SPT was followed with questionnaires every year. At the age of 4.5 year all children, both SPT positive and SPT negative, were invited to a clinical follow up.

At the clinical visits SPT was performed, the eczema was evaluated with SCORAD and a medical examination was made. Samples of blood, urine, faeces, and breast milk (if the child was breastfed) were collected. In Hudiksvall and Linköping saliva samples were collected, with a special hand pump.

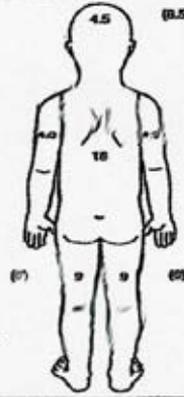
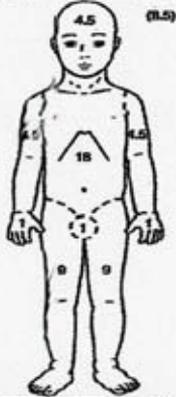
Based on SCORAD European Task Force on atopic dermatitis

Name of assessor Date of visit

Last name First name Date of birth

Topical steroid used Amount/month (G)

Felceve (brand/name) Number of flares/month



Merk the inflamed areas on the diagram and calculate percent of body surface affected.
(Do not include dry but non-inflamed areas)

Figures in brackets for children smaller two years

A: Extent (Please indicate the area involved)

For a typical affected area

B: Intensity

Criteria	Intensity	Means of calculation Intensity items (average representative area) 0 = absence 1 = mild 2 = moderate 3 = severe
Redness	<input type="text"/>	
Swelling or roughness	<input type="text"/>	
Oozing/crust	<input type="text"/>	
Scratch marks	<input type="text"/>	
Thickening of skin and deeper skin creases	<input type="text"/>	
Dryness of unaffected skin	<input type="text"/>	

C: Subjective symptoms pruritus + sleep loss

SCORAD $A/5 + 7B/2 + C$

Visual analog scale (average for the last 3 days or nights)

Pruritis (0 to 10) 0 10

Sleep loss (0 to 10) 0 10

Recommended treatment:

Remarks:

Figure 2: SCORAD form designed by the inventors [93]

Questionnaires

A questionnaire modified from the Swedish Paediatric Allergology Association Questionnaire was used at inclusion. The questionnaires used during follow up were age adjusted with questions about allergy related topics.

Ethics

The study was approved by the Human Research Ethics Committee at the Faculty of Health Sciences, University of Linköping, and the Medical Faculty at Uppsala University.

PAPER II

Study design

This study analyzes results from the first (inclusion visit) and the second visit after 6 weeks regarding the effect of eczema treatment. The time progress in the study is shown in the flowchart in Fig. 3.

Elimination diet

According to praxis at the time of the study at the participating units, children with eczema and positive SPT were recommended to temporarily exclude the corresponding allergen from the diet (and/or from the mother's diet, if the child was breast-fed). Nutritional advice was given by a dietician.

Skin care

All parents were instructed in, and practically shown, skin care with emollients and when needed, topical steroids.

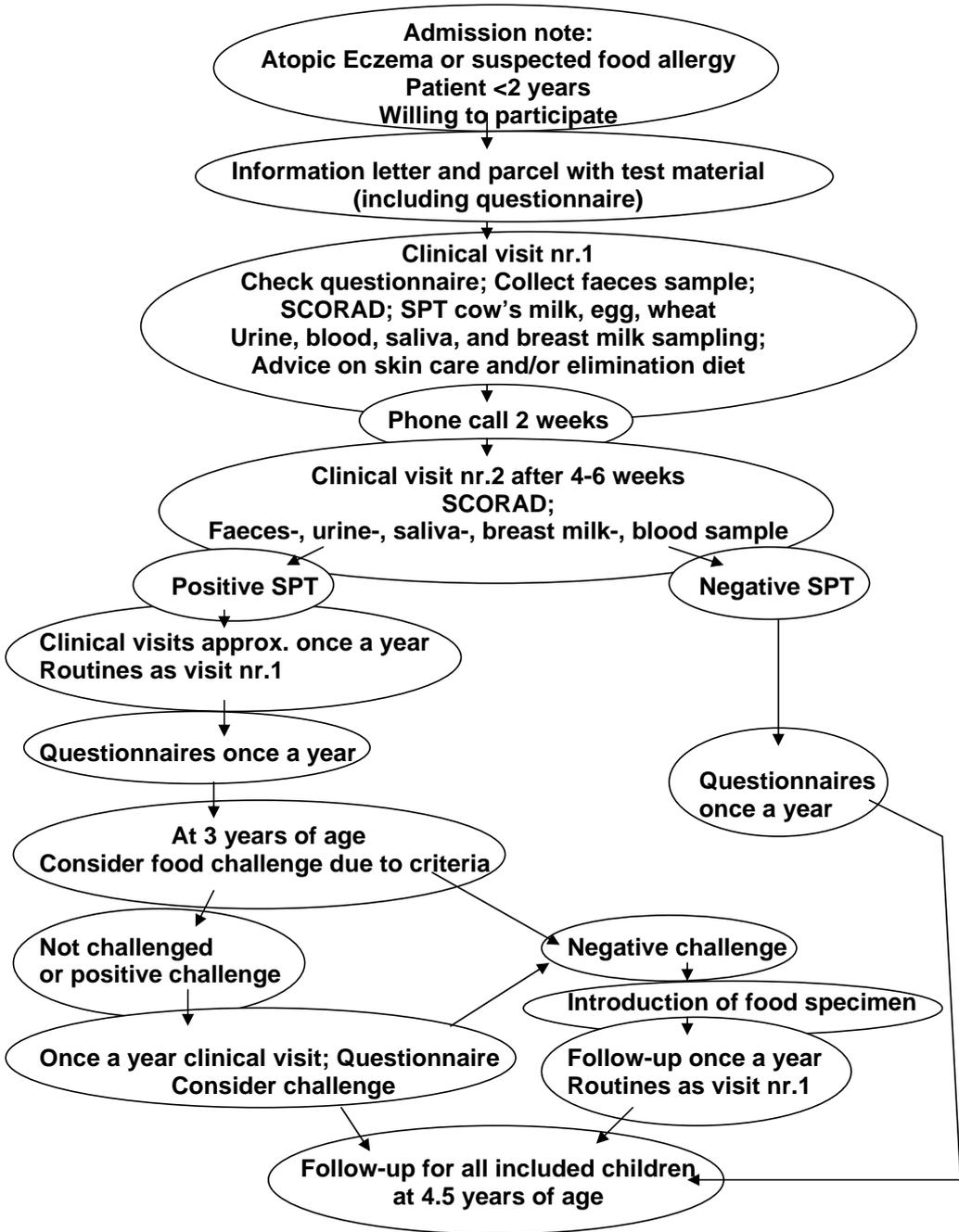
Specific IgE levels

The serum sample from visit 1 were analyzed for levels of specific IgE antibodies to milk and egg with UniCAP[®], a commercial fluoroenzyme immunoassay, according to the recommendations of the manufacturer (Pharmacia Diagnostics AB, Uppsala, Sweden). The test results were considered positive at values ≥ 0.35 kUA/l.

Statistics

Since neither antibody levels nor SCORAD indexes were normally distributed, non-parametric tests were used. Paired analyses were performed with the Wilcoxon signed-rank test and unpaired analyses with Mann-Whitney *U* test. A probability level of <5% was considered statistically significant. The

Figure 3: Flowchart for the eczema/ food reaction study



calculations were performed with a statistical package StatView 5.0 for PC (SAS Institute Inc., Cary, NC, USA).

PAPER III

Study design

This paper analyzes the results from the first, second and the 4.5 year visits regarding changes over time in serum and salivary antibodies. From the population of 123 children, this study includes 93 children with available serum samples from inclusion. Sixty-three children had shown a positive SPT to egg and/or milk at inclusion, and had thus been recommended an elimination diet.

Blood samples

Sera were collected after allowing the blood to clot at room temperature and were then stored at -20°C until analysis.

IgE: were analyzed as described above (paper II).

Specific IgG₁ and IgG₄: IgG₁ and IgG₄ antibodies to BLG and OVA in serum were determined as described earlier, except that blocking was performed with bovine serum albumin (BSA) (Fraction V, Sigma-Aldrich) instead of human serum albumin (HSA). The serum samples were diluted 1:25 to 1:10000. Values were expressed in arbitrary units (AU)/ml deduced from the optical density (OD) of a standard curve after subtracting the blanks. The standard was obtained from an individual with high IgG₁ or IgG₄ antibody titers to BLG and OVA. A coefficient of variation (CV) below 15% was accepted for duplicate samples. A control sample was included in every analysis, and the interassay CV was 21% for IgG₄ to OVA and BLG, 11% for IgG₁ to OVA and 4% for IgG₁ to BLG.

Saliva

Before analysis, the saliva samples were heated in water at 51°C for 30 minutes and then centrifuged at 5000 g for 15 minutes.

Total IgA and total SIgA: Total IgA and total SIgA antibodies in saliva were analyzed as described earlier [94]. A CV below 15% was accepted for duplicate samples. A control sample was included in every analysis, and the interassay was 10% for total IgA and 14% for total SIgA.

Specific IgA: An enzyme amplification system was used to detect of salivary IgA antibodies to OVA and BLG, as described earlier [94], with the exception that all samples were referred to a reference saliva sample with high levels of

IgA antibodies to OVA and BLG and low background. Both the reference sample and saliva samples were diluted 1:25. Uncoated rows were used for individual controls. Antibody levels in the samples were calculated as a ratio between the OD of the sample and the OD of the reference, after subtracting the OD of the blanks and the OD values for the individual controls. The ratio was then expressed in AU. A CV below 15% was accepted for duplicate samples. Two control samples were included in every analysis, and the interassay CV was 25% for IgA to both OVA and BLG.

Statistics

As the antibody levels were not distributed normally, non-parametric tests were used. Paired analyses were performed with the Wilcoxon signed-rank test and unpaired analyses with the Mann-Whitney *U* test. A probability level of <5% was considered to be statistically significant. The calculations were performed with a statistical package, StatView 5.0 for PC (SAS Institute Inc., Cary, NC, USA).

To enable statistical analysis, samples with concentrations below the limit of detection were assigned a value equivalent to half the cutoff value.

PAPER IV

Study design

This study describes the development of recipes and a protocol for low-dose oral food challenge, and the outcome of open and blinded challenges. The study includes 39 children on elimination diet regarding milk and/or egg, who fulfilled the criteria for oral food challenge at three years of age.

Recipes

Recipes suitable for young children were developed for use in both double blind and open standardized oral food challenges. The recipes were tested on a taste panel comprising both adults and children. No detectable difference between placebo and active substance was perceived.

Food challenge criteria

At three years of age the SPT-positive children were suggested food challenge if the SPT diameter was ≤ 10 mm, and SCORAD ≤ 25 and there was no report of severe allergic reaction within the last six months. The challenge was done in a

double-blind-placebo-controlled way in Linköping and Norrköping, and as open standardized in Jönköping and Hudiksvall.

The oral challenge was made in the morning after a light breakfast. Antihistamines had been withdrawn three days previously. The children received the food in ascending doses with 20 minutes interval. Before start the child was equipped with an intravenous access for treatment of a possible anaphylactic reaction, and a physician and nurse were present during the entire process.

The children were observed at the clinic for two hours after the final dose was administered.

In the double-blind challenges the interval between the two sessions was two weeks, and after the two challenges the code was broken. The family was contacted by a nurse the following day and after a week to detect any late reactions. If neither early nor late allergic manifestations were recorded, the family was instructed to reintroduce the food item, in successively increasing doses.

Blood samples

Before the oral challenge blood tests were obtained to measure total and specific IgE. These tests were analyzed retrospectively, and with the same method as described above.

Statistics

For statistical analysis, the Mann-Whitney *U*-test was used. Differences associated with *p*-values of less than 0.05 (2-tailed) were considered significant.

Results and Discussion

Paper I

Skin prick tests

In this study 5.908 patients were investigated with SPT, and altogether 39.705 skin pricks were documented. The numbers of allergen tested on each patient varied from 1-22, mean 6.7. A positive test (*i.e.* at least one skin prick positive) was shown in 46% of the patients. The prevalence of positive SPT ranged from 26% to 59% at the different units. There was a correlation between the number of tests at each unit and the percentage of positive tests (correlation coefficient: 0.86; $p < 0.01$).

Of the total 39.705 skin pricks performed 18% were positive. The distribution of positive tests in different allergen groups is shown in Table 8.

Children with moderate or severe eczema revealed positive SPT to food allergens more often than children with no or mild eczema (RR: 2.29; ci: 2.07-2.54; $p < 0.001$).

Allergen	Food	Pollen	Animal	Various
Percent positive skin pricks	27%	19%	21%	6%

Table 8: Percentage of positive skin pricks in different allergen groups.

Adverse reactions

Sixteen children were recorded with adverse reactions. In seven cases the symptoms were interpreted as GAR. This gives a risk figure of 0.12% for GAR, which is significantly lower than the 0.52% reported in our previous study ($p < 0.005$) [95]. That study was conducted solely in a university hospital, which could suggest a selection of more vulnerable and severely diseased children. Two children displayed LAR. The LAR reactions were considered as exaggerated local reactions to the allergen, and not clinically interesting, and were thus not further analyzed.

Seven children showed VVR, which gives the same risk figure, 0.12%. The risk differs between age groups as shown in Table 9.

The risk factor observed for the youngest children, 0-1 year, was 0.39%, compared to 2.54% in our previous study [95]. This difference is also statistically significant ($p < 0.001$).

In the group with GAR the mean age was 4.1 years, and in the group with VVR 11.1 years.

Among the children with GAR, 5/7 were boys, while in the VVR group 2/7 were boys. In contrast to our findings of a male dominance in children with GAR, there was reported a female dominance in two previous studies [90, 96], whereas another study on fatal reactions show no sex difference [91]. However, these studies included mainly adults.

	GAR	LAR	VVR
Infants (0-2 yrs)	0.24%	0.06%	0.00%
Preschool children (3-6 yrs)	0.07%	0.00%	0.07%
School children (7-12 yrs)	0.10%	0.05%	0.20%
Teenagers (13-18 yrs)	0.00%	0.00%	0.24%
Total	0.12%	0.03%	0.12%

Table 9: Risk figures in different age groups

Risk factors

For GAR there were two identified risk factors: low age (below 1 year) and active eczema. The risk ratio for male sex was not statistically significant due to imbalance in the figures, but it might be clinically important. None of the children with GAR had signs of any infection, which we suspected could be a risk factor when we designed the study. In our previous study [95] SPT with fresh food allergen was considered as a risk factor, but in the present study no particular group of allergen increased the risk for GAR. A previous study by Valyasevi et al [97] focused on the risk for systemic reactions when performing penicillin skin tests, but we did not find any adverse reactions connected to SPT with penicillin. For VVR, to our knowledge no risk factors have been described earlier, even though the reactions are well known in clinical practice. The children with VVR presented two risk factors: female sex and multiple skin pricks on one patient, see risk ratios in Table 10. The patients with VVR had higher median age than those without any reaction and those with GAR ($p < 0.05$). For the child/adolescent and family, a VVR reaction may be perceived just as dramatic as an allergic event, and correct management is important for psychological reasons.

GAR				VVR			
Risk factor	RR	CI	p-value	Risk factor	RR	CI	p-value
Age<1yr	6.28	1.58–25.06	<0.05	Age 13-18 yrs	2.46	0.55–10.95	0.3
Male sex	2.09	0.42–10.38	0.3	Female sex	7.32	1.20-44.41	0.05
Eczema	16.98	4.98-55.21	<0.001	Multiple skin pricks			<0.05

Table 10: Risk ratios for the risk factors presented

Paper II

SPT-results

All 123 children were skin prick tested for cow's milk, wheat and egg at their first visit. The SPT turned out positive for at least one allergen in 76 children (62%). Fifty-nine children had positive SPT to egg, 41 to milk and 9 to wheat. Twenty-six children displayed positive tests for both milk and egg, seven of them showed positive results on all three allergens.

Eczema scoring

SCORAD was performed in 120 children at the first visit, and in 111 children at the second visit (evaluation was missed in three and 12 patients respectively). SCORAD values were distributed between 0 and 77 at the first visit and 0 and 45.2 at the second visit. The children were judged to have mild, moderate or severe eczema according to the SCORAD value as shown in Table 11. Our figures can be compared with a large Swedish study (the BAMSE study) [98] where it also was very few children with severe eczema at inclusion, 2% compared to 8 % in our study.

Infants with positive SPT had higher SCORAD values than those with negative SPT ($p < 0.01$), but there was no difference between these groups after treatment (diet, skin care).

In both SPT-positive and SPT-negative children the SCORAD-indices decreased after the six week treatment period (Fig.4). Improvement of eczema during an elimination period in sensitized children is in line with the major opinion [99-101].

Eczema grade (SCORAD value)	Visit 1	Visit 2
Mild eczema (0-25)	74 (62%)	100 (90%)
Moderate (26-50)	36 (30%)	11 (10%)
Severe (>50)	10 (8%)	0 (zero)

Table 11: Eczema severity at the first and second visit: number and percentage in SCORAD groups

Specific S-IgE

Blood samples were obtained from all 123 children at inclusion. Fifty-seven children had detectable circulating specific IgE for milk or egg: 51 to egg, 30 to milk and 24 to both. 10 children had positive specific IgE, but negative SPT to that allergen. This subgroup was thus not recommended diet. This subgroup had similar levels of SCORAD as the group with positive SPT at inclusion. Interestingly, the SCORAD values tended to decrease more in this subgroup than in the group of children put on a diet (Fig.5). The finding of similar improvement with skin care only contrasts to the findings by Lever et al [101].

Figure 4: SCORAD values at the first and second visit in SPT-negative and SPT-positive children. All children were treated with skin care, and the SPT-positive children also received an elimination diet.

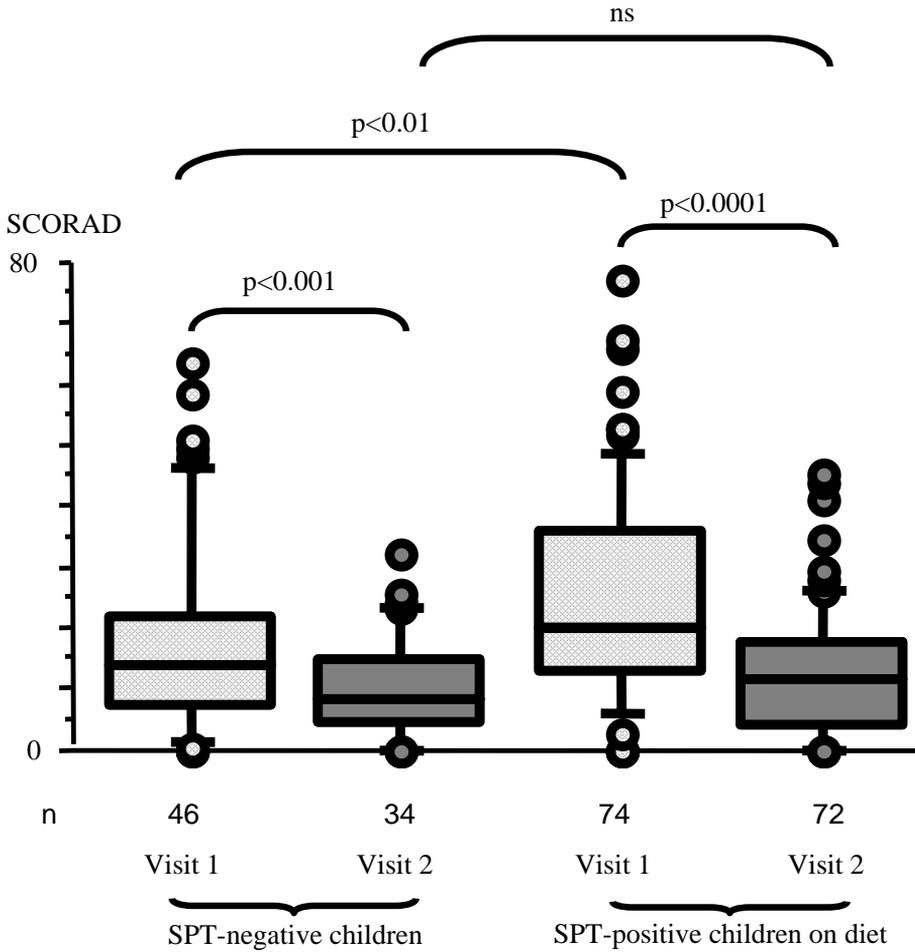
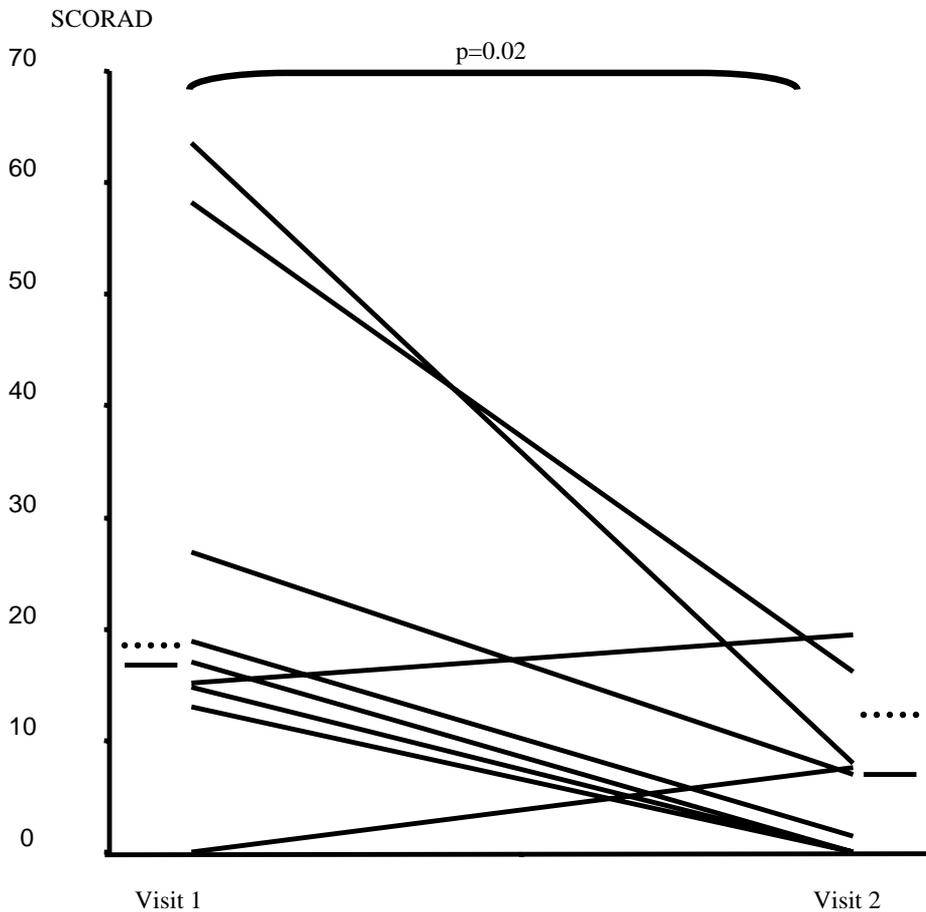


Figure 5. Eczema improvement in 9 children sensitized to milk or egg according to circulating IgE antibodies, but with negative SPT to the same allergen, before and after treatment with skin care and no elimination diet. Median SCORAD values are indicated for these 9 sensitized children with no elimination diet (black line), and for 76 SPT-positive children recommended an elimination diet (dotted line).



Paper III

Antibody levels in relation to treatment regime

The levels of ovalbumin specific IgG₁ and IgG₄ were higher in children with egg positive as compared to egg negative SPT and this difference remained after six weeks treatment for IgG₁-antibodies (data not shown). On the other hand, IgG₄ levels to BLG were equal in milk SPT-positive and negative children at inclusion, but after six weeks treatment the levels were lower in children on a diet (data not shown).

The levels of IgA antibodies in saliva to food allergens, sIgA- and total-IgA did not differ between the two groups at any time point.

SPT-positive children had higher levels of total-IgE and more often detectable levels of specific IgE to food allergens at inclusion and after six weeks treatment.

There were no changes in total or allergen-specific IgE, IgG and IgA levels after six weeks treatment with skin care or food elimination.

Antibody levels in relation to tolerance development

The children who had achieved tolerance to foods at 4.5 years of age had at inclusion higher levels of IgG₄ antibody levels and higher IgG₄/IgE ratios to OVA and BLG than those who remained food intolerant at that age. High levels of food specific IgE has been proposed as a predictor for persistent food allergy [102], but in this study we did not see any difference in the IgE-levels between these two groups. A ratio of IgG₄ and IgE gave a strong association with tolerance development. This indicates that children with high IgG₄/IgE ratios could introduce their offending food earlier, *i.e.* that they have an immune response that reduces prolonged food allergy. Our results are in line with a recent report, indicating low IgE/IgG₄ ratios in children with negative DBPCFC [103].

In the subgroup with negative SPT but positive sIgE, there were even higher ratios of IgG₄/IgE. This could suggest that high levels of IgG₄ compete with IgE for allergen binding to IgE receptor expressing cells, and thereby inhibiting mast cell degranulation [104]. Total IgE, serum IgG₁, and salivary IgA antibody levels did not show any correlation with tolerance development.

At 4.5 years of age the levels of specific IgE were lower in the group which had developed tolerance, whereas the opposite results were found in both IgG₁/IgE and IgG₄/IgE ratios.

Paper IV

Recipes

The recipes and the protocol for standardized open and double-blind placebo-controlled food challenge were well accepted by the children and the staff. There were few difficulties convincing the children to eat and drink the samples, which can often be the case in a challenge procedure that requires greater amounts or use of unnatural or unfamiliar forms.

Cut off values

The size of SPT and concentration of IgE are commonly used tools to decide whether to challenge or not. The traditional cut-off level (≤ 3 mm) has a great potential of diagnostic error [105]. Sporik et al [88] suggest 8 mm for milk and 7 mm for egg, as cut-off levels for challenge. Cut-off levels at 5 mm, and 4 mm respectively for milk and egg are suggested by Thong et al [80]. Niggeman et al [106] proposes 12.5 mm for milk and 13 mm for egg. Cutaneous reactivity though varies with age, time of day, season and gender, so different cut-off values are therefore likely to be required in different subpopulations of children [79, 105, 106].

Food challenge

There was a positive result in four out of 52 challenges. These four children reacted with immediate allergic symptoms early in the procedure (first to third dose). All were subjected to DBPCFC with milk. One of them had a negative SPT before challenge, but expressed a SPT with 10.5 mm diameter two weeks after challenge.

There was no relationship between the size of the SPT ($p=0.12$), or the SCORAD value ($p=0.40$) and the outcome of challenge. Neither there were any differences regarding family history, atopic manifestations or breastfeeding. However, there was an association between total, and food-specific IgE and challenge outcome ($p<0.005$ and $p<0.01$ respectively). The blood samples were analyzed retrospectively in our study, and were therefore not considered as criteria before challenge. As this was a rather limited group, one should be cautious in interpretation of results, especially as recent studies has shown diverging results.

Post food challenge follow-up

Three months later three of the four children with positive food challenge were still on a diet. All but two of the children with negative food challenge had

successfully introduced the respective food item into their diet. One of them who had not reintroduced was a twin with an extremely allergic twin brother, and the other one had gastrointestinal symptoms from milk which were diagnosed as non-IgE mediated hypersensitivity. In some of the non-reacting children who had introduced milk and/or egg, the parents reported episodes of aggravated eczema. This was interpreted as a normal fluctuation of eczema, and did not interfere with the reintroduction schedule.

Because tolerance develops gradually, parents may notice that their child can tolerate small amounts of the food, especially in cooked form, even if greater amounts still gives symptoms [79], and this favours the family's quality of life, as well as the child's nutrition and growth [107].

Evaluation of the model

Our model for low-dose challenge can facilitate early re-/introduction of food (milk and egg) in young children outgrowing their allergy. The recipes for blinded challenge can also be useful for older children with a strong emotional expectation regarding outcome of the food challenge.

Nitric oxide (NO) and eczema (unpublished)

The aim of this study was to assess the levels of urinary NO breakdown products in children with eczema, and to evaluate whether there is a correlation between an effect of eczema treatment and NO formation.

Nitric oxide (NO) and reactive nitrogen products have been implicated in the pathogenesis of inflammatory diseases [108]. NO formation is catalyzed by NO synthases (NOS), two constitutive (cNOS), and one inducible form (iNOS). The constitutive ones produce low amounts of NO and have generally been associated with the regulation of homeostatic functions. iNOS, on the other hand, produces large amounts of NO and is induced in various cells by inflammatory stimuli, and has been linked to severe tissue damage [109]. NO reacts rapidly with oxygen, yielding nitrite and nitrate, which are excreted into the urine.

In asthma, the level of NO in exhaled air is frequently used for monitoring the inflammatory status of the bronchial epithelium [110]. Traditionally, NO has been considered to act mainly as a pro-inflammatory agent [111], but this concept has recently been challenged in a study on human bronchial epithelium, suggesting that NO is also involved in an anti-inflammatory feed-back loop [112].

Less is known about the role of NO in eczema. In an animal model using NC/Nga mice to simulate human eczema, NO breakdown products were increased in serum, but decreased in the skin lesions, compared to control animals [113].

Previous studies measuring NO products in children with eczema have shown diverging results, and analyses have been performed both in serum and urinary samples. Taniuchi et al found increased serum levels of nitrite/nitrate, which decreased upon eczema treatment [114]. However, when measuring urinary nitrite/nitrate levels, Omata et al showed decreased levels of nitrite/nitrate in eczematous children, and no relation between the levels and eczema severity [115]. In young children, urinary sampling is far more convenient than blood sampling.

Patients

The study group comprised 94 children (58 boys, 36 girls) below two years of age from the prospective study of eczema in infants (same population as paper II-IV). They were examined twice, with a 6-8 week interval, and for this study

we chose children with urinary samples and SCORAD assessments from both visit [116].

According to Swedish recommendations for infants, baby food should not contain any food items with high levels of nitrite/nitrate, and these recommendations were given to the mothers at the well-baby clinics both orally and in a pamphlet about good baby feeding.

Nitrite/nitrate measurement

In the urinary sample, the sum of nitrite and nitrate was measured and taken as an indirect indicator of the NO production [117]. In short, the nitrite content was assessed with a colorimetric method based on Griess reaction for nitrite. In a PBS-diluted sample, nitrate was converted using nitrate reductase from *Aspergillus* [118]. Next, 50 μ l of the diluted urine was mixed with 10 μ l NADPH (1 μ M) followed by 40 μ l containing nitrate reductase (80 U/l, Roche, Basel, Switzerland), glucose-6-phosphate (500 μ M) and glucose-6-phosphate dehydrogenase (160 U/l). The reaction mixture was incubated at room temperature for 45 min. The mixture was then used for the Griess assay of nitrite by adding 100 μ l sulfanilamide (1% in 5% phosphoric acid) and 100 μ l naphthylethylenediamine (0.1 %). The resultant colour was read with a spectrophotometer (Vmax, Molecular Devices, Sunnyvale, CA) at 540 nm.

Statistics

For statistical analysis, the Mann-Whitney *U* test was used to compare groups. Differences associated with p-values of less than 0.05 (2-tailed) were considered significant. Pearson's test was used to evaluate correlations.

Results

The SCORAD value decreased significantly from the first to the second visit; 22.0 ± 6.0 ; 17.1; 0-77 (mean \pm SD; median; range) vs 11.6 ± 10.4 ; 9.0; 0-45.2 ($p < .001$).

The urinary nitrite/nitrate levels increased significantly from the first to the second visit, 420 ± 428 ; 286; 65-3174 μ M (mean \pm SD; median; range) to 711 ± 775 ; 448; 36-4648 μ M ($p < .001$). There was no correlation between nitrite/nitrate levels and eczema severity neither at the first nor at the second measurement.

Discussion

Considering the inflammation of the skin noted in eczema, we were expecting decreasing values of NO urinary products in parallel with eczema recovery and suppressed inflammation. Contrary to our expectations, the NO products increased significantly after eczema treatment, and there was no significant correlation between eczema severity and NO values. This is in contrast to a previous study of children with eczema and the effect of treatment [114]. In that study, the children showed significantly increased levels of serum nitrate compared with healthy children and lower nitrate levels after eczema treatment. Moreover, the serum nitrate levels correlated with the severity of the eczema [114]. However, our results corroborate with those of Omata et al, also using urinary measurements, reporting lower levels of urinary nitrite/nitrate in children with eczema and no correlation to eczema severity [115].

In the present study, the urinary excretion of NO products increased significantly after treatment, from $420 \pm 428 \mu\text{M}$ (mean \pm SD) on the first occasion to $711 \pm 775 \mu\text{M}$ at the second investigation. A possible explanation for increasing levels could be increased intake of foods rich in nitrite/nitrate, untreated asthma, steroid treatment of the eczema or age-related increase. We find these explanations unlikely, since the mothers had the same instructions regarding diet, and no difference was seen between children with and without airway problems. Moreover, the majority of children were treated with local steroids already at the first visit, and a previous study has shown that urinary nitrite/nitrate levels decrease with age [119].

In a previous study, we found that the levels in healthy children of the same age were $1174 \pm 116 \mu\text{M}$ (mean \pm SEM, n=53) [120]. In comparison, the levels of nitrite/nitrate in the children with eczema in this study were clearly lower, especially before treatment, but also at the second sampling after eczema treatment, but now they were approaching the levels seen in healthy children.

The immune modulating effects of NO have recently been studied in asthma by *in vitro* studies of human bronchial epithelial cells [112], and these cells were able to up regulate NOS, which causes augmented NO release. It was also previously shown that iNOS increased after ovalbumin challenge in ovalbumin sensitized wild-type mice [121].

Although the above information obtained on human bronchial epithelial cells has primary relevance for asthma, we consider it plausible that a similar mechanism might be operative in eczema as well. Thus, we hypothesize that our findings with low urinary levels of NO in children with active eczema and increased levels after treatment might be explained by a mechanism involving

an up regulation of NOS, as observed in human epithelial cells in asthma. Furthermore, the results support previous studies indicating that increased oxidative stress and impaired homeostasis of nitrogen radicals are associated with childhood eczema.

Conclusions

- Skin prick test in children and adults is a safe procedure, with generalized allergic reactions and vasovagal occurring infrequently. Risk factors for allergic reactions are age below 1 year and active eczema. Female sex and multiple tests are risk factors for vasovagal reactions.
- Sensitization to milk, egg, and wheat was present in the majority of children below 2 years of age with eczema. Treatment for six weeks, *i.e.* skin care for all and elimination diet for food skin prick test positive children, caused a significant reduction of eczema severity. Before treatment, the eczema score was higher among the skin prick test positive children compared to the skin prick test negative ones, but after treatment did the groups display similar eczema severity. A sensitized subgroup, treated only with skin care, also improved their eczema significantly, implying that continued exposure to allergen may not worsen the eczema if there is good skin care.
- Children sensitized to egg and/or milk who had developed tolerance at 4 ½ years of age displayed higher levels of IgG₄ antibodies to ovalbumin and β-lactoglobulin and also higher IgG₄/IgE ratios on inclusion in the study, than those who remained non-tolerant. The highest IgG₄/IgE ratios were found in the subgroup of sensitized, but SPT-negative children. The six-week treatment period did not significantly affect the levels of serum and salivary antibodies.
- Recipes for masking of cow's milk and egg in open or blinded food challenges may help to accomplish challenges in young children, often suspicious to unfamiliar tastes or textures. There was no significant relation between SPT size or SCORAD-value and the outcome of the challenge, but there was a significant correlation between total and food-specific IgE and challenge outcome.

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