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Assessing eczema and food allergy in young children

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CONTENTS

ABSTRACT

Background: Atopic disease is an increasing problem. Eczema affects 10-20% of young children, and 33-37% of children with eczema are food allergic. Among other factors, nitric oxide (NO) is thought to play a role in eczema and food allergy. Following the atopic march, approximately 80% of children with atopic eczema will become sensitized to aeroallergens and develop asthma and/or allergic rhinitis. Skin prick test is used for investigating sensitization and is considered a safe method. However, systemic allergic reactions may appear when the test is performed. In diagnosing food allergy and for evaluating achievement of tolerance, the oral food challenge is the method of choice, and the double-blind placebo-controlled fashion is 'the gold standard'.

Skin prick test: We examined six cases of generalized allergic reactions in connection with skin prick testing in order to identify risk factors, and thereby increase safety, and we investigated the necessity of performing skin prick tests in duplicate. We found that all six children with generalized reactions were <6 months of age. When analyzing skin prick tests in duplicate, we found only 1.3% that showed diverging results, and in infants <6 months even fewer, 0.9%.

Food challenge: We developed recipes and a protocol for low-dose oral food challenge to milk and egg to be used in young children outgrowing their food allergy so as to facilitate early re-/introduction of small amounts of milk and egg. We performed 52 challenges, both open and double-blind placebo controlled. The recipes were validated for blinding. The low-dose challenge was tolerated well by the children and was easy to perform. Four children had a positive challenge outcome, all reacting to very small amounts of milk. All but two of the non-reacting children were able to introduce milk and egg into their diet.

Nitric oxide and eczema: We investigated the effect of eczema treatment on the NO levels in urine. The sum of nitrite and nitrate was measured in urinary samples from 94 infants at two visits, with an interval of 6 weeks, and the results were compared with clinical data. The levels of NO products increased significantly when the eczema improved.

The atopic march: The aim was to evaluate the atopic march in children with eczema, from referral at <2 years until 4½ years of age. We followed 123 children with eczema, 78 sensitized and 45 not sensitized to milk and/or egg, with respect to eczema severity, other allergic manifestations, development of airway sensitization, and achievement of food tolerance. The difference in severity of eczema at referral was significant when comparing food-sensitized with non-sensitized children. At follow-up, 62% were still affected by eczema, although 56% only mildly so. Tolerance was achieved in 81% of the children allergic to milk and 68% of those allergic to egg. Fifty-eight percent of the food-sensitized children and 26% of the non-sensitized children had become sensitized to aeroallergens, a significant difference. The difference in airway symptoms was not significant. Very few children were exposed to tobacco smoke in their homes.

Conclusions: Increased precautions should be considered when performing skin prick tests in infants <6 months of age. The use of a single prick, to avoid the risk of summation of reactions, is justified when performing skin prick tests. We report recipes and a protocol for standardized open and double-blind placebo-controlled low-dose food challenge in young children, enabling the introduction of small amounts of egg and milk into the diet during tolerance development. NO products in urine increases when eczema improves. This might be due to a Th2/Th1 shift induced by the eczema treatment and skin healing, and the variation in NO response may be due to individual variations in NO-induced feedback downregulation of Th1 and Th2 proliferation. The prognosis for achieving clinical tolerance is very good in children early sensitized and allergic to milk and egg, but they will become significantly more often sensitized to aeroallergens.

LIST OF ORIGINAL PAPERS

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

I Skin prick tests may give generalized allergic reactions in infants.
Devenney Irene and Fälth-Magnusson Karin.
Ann Allergy Asthma Immunol 2000;85:457-460.

II Skin prick test in duplicate: is it necessary?
Devenney Irene and Fälth-Magnusson Karin.
Ann Allergy Asthma Immunol 2001;87:386-389.

III A new model for low-dose challenge in children with allergy to milk or egg.
Devenney Irene, Norrman Gunilla, Oldaeus Göran, Strömberg Leif and Fälth-Magnusson Karin.
Accepted for publication in Acta Paediatrica

IV Nitric oxide urinary products in infants with eczema.
Devenney Irene, Norrman Gunilla, Forslund Tony, Fälth-Magnusson Karin and Sundqvist Tommy.
Submitted.

V Eczema in infancy and the atopic march.
Devenney Irene, Norrman Gunilla, Oldaeus Göran and Fälth-Magnusson Karin.
In manuscript.

ABBREVIATIONS

AE	atopic eczema
AR	allergic rhinitis
CD	celiac disease
cNOS	constitutive nitric oxide synthase
DBPCFC	double-blind placebo-controlled food challenge
EAACI	European Academy of Allergology and Clinical Immunology
IFN-γ	interferon gamma
IgE	immunoglobulin E antibody
IL	interleukin
iNOS	inducible nitric oxide synthase
kU_A/L	kilo unit antibodies per liter
LPS	lipopolysaccharide
MBP	major basic protein
mRNA	messenger ribonucleic acid
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
NPA	negative predictive accuracy
NPV	negative predictive value
PEG	polyethyleneglycol
PPA	positive predictive accuracy
PPV	positive predictive value
RAST	radioallergosorbent test
SCORAD	Severity Scoring of Atopic Dermatitis
sIgA	secretory immunoglobulin A
SPT	skin prick test
TGF-β	transforming growth factor beta
Th1	CD4+ T helper cell type 1
Th2	CD4+ T helper cell type 2

DEFINITIONS AND EXPLANATIONS

Allergen	A foreign protein which induces formation of IgE-antibodies. Substances such as foods, pollen, mites and animal danders are allergen sources, but often referred to as allergens. Each allergen source contains many different allergenic proteins, each being an allergen.[1]
Allergy	Hypersensitivity reaction initiated by specific immunological mechanisms.[2]
IgE-mediated allergy	Allergy mediated by antibodies belonging to the IgE isotype.[2]
Atopic symptoms	Allergic symptoms in a person of atopic constitution.[2]
Atopy	A hereditary predisposition to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins. As a consequence, these persons can develop typical symptoms of asthma, rhinoconjunctivitis or atopic eczema.[2]
Hypersensitivity	Objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons.[2]
Sensitization	The immunological events leading to IgE antibody formation against a particular allergen. Once sensitized, the person is predisposed to develop allergic inflammation and allergic disease upon reexposure to the same allergen.[1]
T cell responses	T cells regulate and organize most types of immune responses to foreign proteins by secreting cytokines such as interleukins (IL) and interferons (IFN). The T helper (Th0) cells are differentiated in Th1 and Th2 cells, classified according to the predominant profile of their cytokine production. The Th2 initiate the immediate allergic response by releasing IL-4, IL-5, IL-10 and IL-13. These cytokines, by IL-4 and IL-13, stimulate the maturation of B-cells and initiate their production of IgE antibodies. The Th2-derived pro-inflammatory cytokines also induce tissue eosinophilia and promote the growth of mast cells. By contrast, Th1 cell responses protect against microbial infections, are primarily involved in chronic inflammation and have been proposed to inhibit Th2-driven processes. Genetics and environmental factors could modify the T-cells and either increase the risk of developing sensitization or promote the development of tolerance.[3, 4]

INTRODUCTION

Eczema and food allergy were first described more than two thousand years ago. Hippocrates used the word *eczema* (*ek*, out, *zeo*, boil), and the Bible's Old Testament (Lev. 14:1-57) describes rituals for treating skin diseases. In 1483, Sir Thomas More recounts how King Richard III sentenced an English nobleman to death on a charge of using witchcraft to induce a skin rash on the royal person. In fact, according to More, the rash occurred after the king had eaten and reacted to strawberries.[5] The first *anaphylactic reaction* to egg was recorded by Marcello Donati in the sixteenth century.[6]
The term and concept of *allergy* (from the Greek *allos*, other, and *ergon*, reaction) was coined by a Viennese pediatrician named Clemens von Piquet in 1906. He observed that the symptoms of some of his patients might have been a response to outside allergens such as dust, pollen, or certain foods.[5] In 1918, Talbot described *food allergy* in a series of patients with eczema and positive skin tests to foods who significantly improved after elimination of the suspected foods from their diet.[7]

The term *atopy* was first introduced by AF Coca and RA Cooke in 1923 to describe a state of hypersensitivity characterized by immediate-type wheal reaction, allergic manifestations such as asthma, eczema and hay fever, and circulating reagins[5], and in 1950 MH Loveless was the first to use *blinded placebo-controlled food challenges* to establish the diagnosis of food allergy and to demonstrate the unreliability of patients' history.[8]

Although reference has been made to eczema and food allergy for more than two millennia, it is only in modern times that the literature indicates that these conditions have successively become more prevalent. For instance, the prevalence of atopic eczema has increased two- to three-fold in developed countries during the past three decades.[9] Eczema affects 10-20 % of young children at least transiently and most often represents the first manifestation of atopy in infancy.[9,10]
The prevalence of food allergy in childhood today is 6-8% and peaks at one year of age. It then falls progressively until late childhood, after which the prevalence remains stable at 1-2 %.[11-13] Of children with eczema, 33-37% have food allergy.[14-16] Food allergic reactions account for 20% of acute urticaria and for a third of moderate to severe eczema in children. Food allergy is also the most common cause of anaphylaxis, 25-60% of all cases.[17] During infancy the most common offending foods are cow's milk and hen's egg.[18]

THE ATOPIC MARCH

The "atopic march" refers to the natural history of atopic manifestations, which is characterized by a typical sequence of sensitization to common environmental allergens and clinical allergic symptoms, which appear during a certain age period, persist over years and decades, and often show a tendency toward spontaneous remission with age.[18] Within the atopic march, atopic eczema (AE) and food allergy are often the first manifestations of atopy and identify an individual destined to a lifetime of allergy and asthma.[9] Food allergy most often begins in the first 1 to 2 years of life with the process of sensitization, by which the immune system responds to specific food proteins with the development of allergen-specific IgE. Once sensitized, the allergic individual may experience an adverse reaction on repeated exposure to a sufficient dose of that food.[12]

As the atopic march continues, most allergies to foods are outgrown, typically in early childhood.[9] The loss of food allergy is, however, a variable process, depending on both the individual child and the specific food allergy. For instance, whereas milk and egg allergies are outgrown in about 80 % of children before school-age, allergies to peanuts and tree nuts are rarely lost.[12] Following the atopic march approximately 80% of children with AE will become sensitized to aeroallergens and develop asthma and/or allergic rhinitis, with many outgrowing their AE with the onset of respiratory allergy.[9,19,20] The sensitization to aeroallergens may precede the airway symptoms by months or years, whereas some sensitized children will never develop any clinical allergic airway manifestation.

Risk factors in atopic disease

Genetics, exposure to tobacco smoke and allergens, damp houses, lifestyle, and infections are some of the factors suggested to affect the course of the atopic march in the individual child.

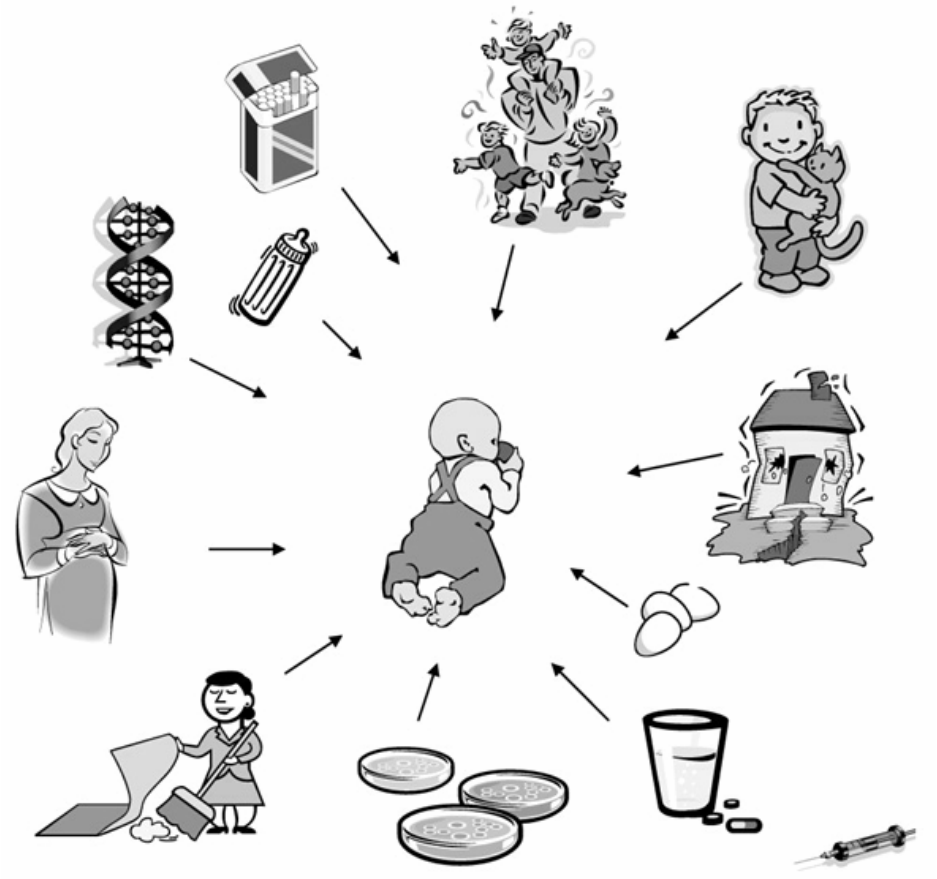
The risk of the child becoming atopic and developing atopic diseases is strongly associated with the manifestations of the disease in the parents and siblings. Genetic factors account for around 50% of atopic diseases.[20] There is a closer association between specific symptoms, such as asthma and AE, in the child and the same manifestations in parents or siblings than with other atopic manifestations in the family. These clinical observations suggest the presence of phenotype-specific genes. There is also an indication that the influence of the maternal phenotype on the development of the disease in children is stronger than that of the paternal phenotype.[18]

With respect to immunological markers, elevated cord blood IgE concentrations has been shown to correlate with later development of atopic disease.[21] However, more recent studies demonstrate the elevated levels to be capable of predicting early sensitization, but not airway and skin symptoms.[18] High levels of eosinophil cationic proteins in wheezing infants compared with control infants have been shown, but not the capacity of elevated levels to predict asthma.[18] However, strong infantile IgE antibody responses to food proteins are predictors of subsequent sensitization to

aeroallergens.[18] In vitro responses of cord blood mononuclear cells to allergen stimulation have been demonstrated to differ between infants in whom atopic diseases develop later on and in healthy controls, showing reduced capacity to secrete IFN-γ in the atopic infants.[18] Low Th1 cytokine production at 12 months has also been associated with atopic sensitization to aeroallergens at 6 years of age.[18]

The role of so-called western lifestyle (small family size, increased income and education, migration from rural to urban environments, changed food habits, and increased use of antibiotics) as a risk factor is supported by studies in which allergic responses were shown to be driven by Th2 immune responses, whereas infections induce the Th1 immune responses. Since Th1 responses antagonize the development of Th2 cells, a decreased number of infections or the absence of Th1 polarizing signals (such as endotoxins) during early childhood could predispose children to enhanced Th2 allergic responses.[9]

Tobacco smoke increases the risk of recurrent wheezing in infants and has been shown to affect respiratory health in children of all ages, with high cotinine levels being associated with asthma in children aged 4 years and older.[22] An increased sensitization to food proteins in infants of smoking mothers has also been demonstrated.[18]



Prevention of atopic disease

If preventive inventions are to be effective at all, they would have to be applied early in life, most probably in early infancy. Our understanding of the natural history of the atopic march is still limited, and many unknown factors may play a role. Therefore possible primary preventive measures recommended should be applicable to the whole population, riskfree, and at low cost.[18]

The aim of primary prevention must be to prevent the development of disease and not only sensitization.[23]

- Breastfeeding should be encouraged for 4-6 months.[23,24,25] If supplement is needed, conventional cow's-milk-based formula is recommended for infants without a high risk of allergic disease.[23] Solid foods should be avoided the first 4-6 months.[23] No special diet is recommended during pregnancy or to the lactating mother.[23]
- In infants with increased risk of developing allergic disease, i.e. at least one first-degree relative with documented allergic disease, a documented hypoallergenic formula is recommended during the first 4-6 months.[18,23,*] After the age of 4-6 months, high-risk infants can be nourished like non-high-risk children.[23,24]
- Because early exposure to tobacco smoke is associated with recurrent wheezing in childhood, asthma at preschool age and the risk of developing IgE responses to food proteins early in life, smoking should always be avoided.[18,22,24]

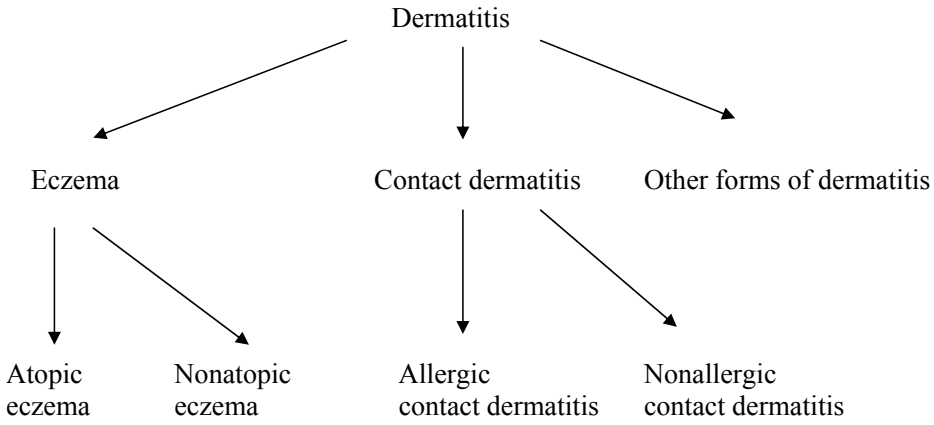
Measures for secondary prevention are aimed at 'high-risk' infants, i.e. children with a positive family history of atopy in first-degree relatives and who have displayed AE or sensitization to food protein in infancy.[18]

- Reduction of allergen exposure by using mattress encasings, avoiding damp housing conditions, and avoiding furred pets at home is also recommended in high-risk children.[18]
- Pharmacological intervention with ceterizine has been demonstrated in studies to lead to a lower incidence of asthma in children early sensitized to grass pollen or dust mites.[18,26]
- Allergen-specific immunotherapy has been shown to reduce the incidence of seasonal asthma.[18]

* The Swedish group of Pediatric Allergologists (Svenska Barnläkarföreningens allergisektion) recommends hypoallergenic formula for infants < 4 months of age if there are two or more family members with severe allergic disease that requires treatment.[27]

ECZEMA

The terminology used for characterize *eczema*, *allergic* and *non-allergic* has long been confusing. The nomenclature proposed by EAACI nomenclature task force in October 2003 is based on the mechanisms that initiate and mediate allergic reactions and aims to clarify these skin conditions. The term *eczema* replaces the former term *atopic eczema/dermatitis syndrome* and *atopic dermatitis* (AEDS and AD) and *atopic eczema* (AE) means eczema in a person of the atopic constitution.[2]



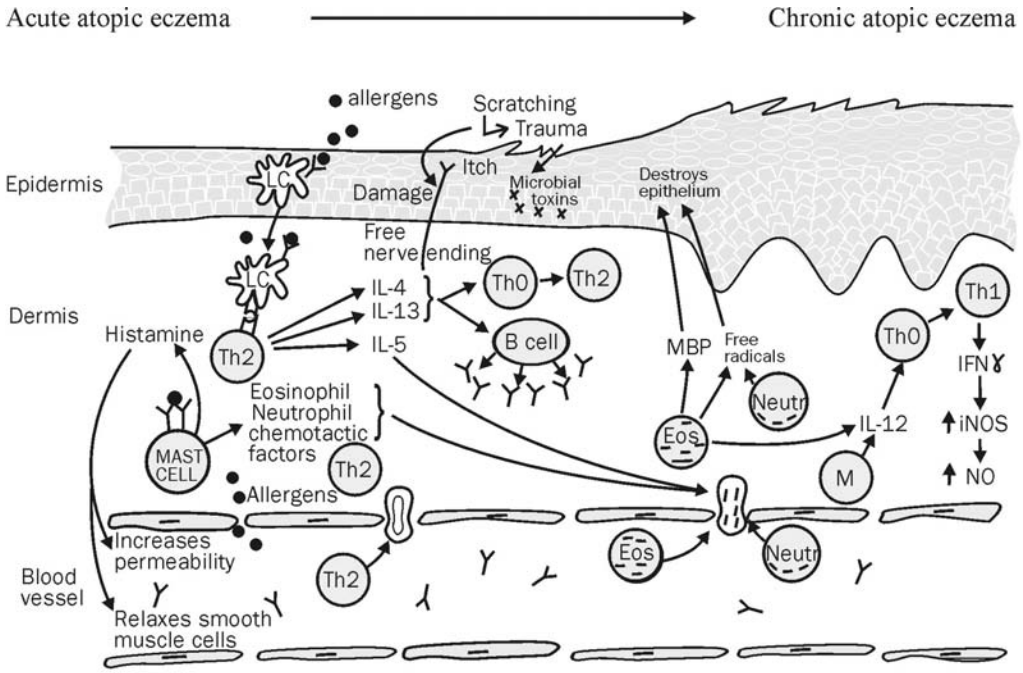
Genetics

AE is the result of an interaction between genetics, the host's environment, pharmacological abnormalities, skin barrier defects, and immunological factors.
The genetics are complex, with a stress on the maternal influence. Parental AE confers a higher risk to offspring than does parental asthma or allergic rhinitis, suggesting the existence of genes not only for atopy, but specific to AE.[9]

Skin pathology

Even clinically unaffected skin in patients with AE is abnormal, with mild hyperkeratosis and a sparse perivascular T-cell infiltrate. Acute eczematous skin lesions are characterized by spongiosis (epidermal intercellular oedema) and increased numbers of antigen-presenting cells (Langerhans cells, inflammatory dendritic epidermal cells and macrophages) bearing IgE molecules. In the dermis with acute lesions, there is a striking infiltration of activated CD4+ T cells. Chronic lichenified lesions are characterized by an acanthotic epidermis, with elongated rete ridges and parakeratosis, but only a minimum of spongiosis. In the epidermis and dermis these

chronic lesions have increased numbers of IgE-bearing Langerhans cells and mast cells, and macrophages dominate the dermal mononuclear cell infiltrate. The chronic lesions also contain eosinophils, which are thought to contribute to inflammation and tissue injury through production of reactive oxygen intermediates and proinflammatory cytokines, and release of toxic eosinophil major basic protein (MBP).[9,19]



Eos = Eosinophils, Neutr = Neutrophils, M = Macrophages, Y = IgE antibody, LC = Langerhans cells, NO = nitric oxide, iNOS = Inducible NO synthase, MBP = Major basic protein, Th = T helper cells, IL = interleukins, IFN-γ = interferon gamma

Immunopathology

Most patients with AE, approximately 85%, have peripheral blood eosinophilia and increased serum IgE concentrations, and about 85% of these have specific IgE antibodies to foods or aeroallergens. In the peripheral blood an increased number of Th2 cells produce IL-4, 5 and 13, but the capacity of mononuclear cells to produce IFN-γ and IL-12 is decreased, which is inversely correlated with serum IgE. The IL-4 and 13 are the only cytokines that promote isotype switching to IgE. In addition, these cytokines also down regulate the Th1-type cytokine activity.

In the atopic skin, even when unaffected by eczema, the numbers of Th2 cells expressing mRNA of IL-4 and 13 are increased compared with non-atopic skin. In

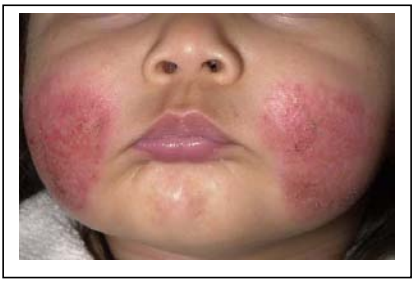
acute AE the cells expressing mRNA of IL-4, 5 and 13 increase, but not the numbers of cells expressing mRNA of IFN-γ or IL-12. By contrast, in the skin of chronic AE fewer cells express mRNA of IL-4 and 13, but more cells express mRNA of IL-12 and IFN-γ, compared with the skin of acute AE. The expression of IL-12 by eosinophils and macrophages is thought to play a key role in the switch to Th1 cells, which are prominent in chronic AE. The antigen-presenting cells have an important role in allergen presentation to Th2 and Th1 cells. High-affinity receptors for IgE contribute to the capture and internalization of allergens before their processing and antigen presentation to the T cells in the atopic skin. These cells can process both allergens entering through the eczematous skin, but also food and aeroallergens that enter the circulation through the gastrointestinal and respiratory mucosa and reach the skin by way of circulation. Also IgE-bearing dendritic cells and macrophages with allergens acquired in the respiratory and gastrointestinal tract may circulate to the skin and activate local T cells. In addition, Langerhans cells positive to these receptors can migrate from the skin to the lymph nodes and stimulate naive cells, thereby contributing to expansion of the pool of Th2 cells. [9,19]

Clinical features of atopic eczema

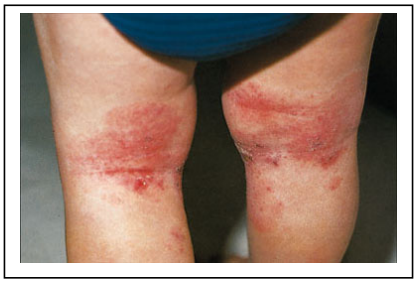
In early infancy the course of the eczema is generally more acute or sub-acute. The skin lesions are characterized by intensely pruritic erythematous papules, excoriations and exudative areas with crusts. It mainly affects the face (especially the cheeks and the chin), scalp, trunk and extensor surfaces of the extremities. The diaper area is commonly spared.[16]

In later childhood the eczema has a more chronic course and presents with lichenification, papules and excoriations, affecting neck, wrists, ankles, and flexural folds (such as antecubital and popliteal fossae) of the extremities.[16]

A number of features are associated with AE, including: early age of onset, xerosis, Dennie-Morgan infraorbital folds, orbital darkening, facial erythema, cheilitis, food intolerance, positive SPT and/or raised serum IgE concentrations, and the influence of environmental and emotional factors.



Facial eczema in the infant



Flexural fold eczema in the child

Diagnostic criteria for atopic eczema

Several diagnostic criteria have been proposed for atopic eczema, such as the Hanifin and Rajka criteria, the Schultz-Larsen criteria, the Danish Allergy Research Centre criteria, and the U.K. Working Party's diagnostic criteria. Agreement is good between the different criteria but less acceptable when comparing them with doctor-diagnosed visible eczema. The Hanifin and Rajka criteria have a high degree of specificity. [28]

DIAGNOSTIC FEATURES OF ATOPIC ECZEMA

(modified for young children from The Hanifin-Rajka Diagnostic Features of Atopic Dermatitis [29])

The diagnosis for atopic eczema requires at least 3/4 basic features:

- Pruritus
- Typical morphology and distribution
  - Facial and extensor involvement in infants
  - Lichenification, papules and excoriations, affecting neck, wrists, ankles, and flexural folds in later childhood
- Chronic or chronically-relapsing eczema
- Personal or family history of atopy

and 3 or more minor features:

- Xerosis: generalized dry skin.
- Immediate (type 1) skin test reactivity
- Elevated levels of serum IgE
- Early age of onset
- Tendency towards cutaneous infection: e.g. Herpes simplex and Staph. Aureus.
- Cheleitis: 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

SCORAD

The Severity Scoring of Atopic Dermatitis (SCORAD)[30] is a standardized method for assessing the eczema, taking into account the extent and severity as well as the consequences of the skin disorder (degree of pruritus and sleeping disorder assessed by the parents). According to this classification, the eczema can be classified as mild (SCORAD ≤25 points), moderate (SCORAD 26 - ≤ 50 points), or severe (SCORAD >50 points) eczema.

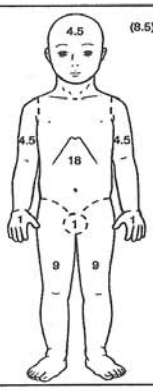
Based on SCORAD European Task Force on atopic dermatitis

Name of assessor | Date of visit

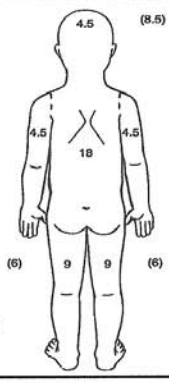
Last name | First name | Date of birth

Topical steroid used | Amount/month (G)

Potency (brand name) | Number of flares/month



Figures in brackets for children under two years



Mark the inflamed areas on the diagram and calculate percent of body surface affected.

(Do not include dry but non-inflamed areas).

A: Extent (Please indicate the area involved)

For a typical affected area

B: Intensity

Criteria	Intensity
Redness	
Swelling or roughness	
Oozing/crust	
Scratch marks	
Thickening of skin and deeper skin creases	
Dryness of unaffected skin	

Means of calculation

Intensity items (average representative area)

0 = absence

1 = mild

2 = moderate

3 = severe

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)

Recommended treatment:

Remarks:

Treatment of eczema

In AE the disturbed function of the skin barrier is probably the result of reduced ceramide concentrations, and results in xerosis and enhanced transepidermal water loss. Irritants such as soaps or detergents can worsen the xerosis. The xerosis contributes to development of epithelial microfissures and cracks, which allows entry of skin pathogens, irritants and allergens.[9]
The local treatment of AE should aim at relieving all the different elements of the skin disease, such as the xerosis, the inflammation, the itch, secondary skin infections, and also to reduce the number of precipitating and aggravating factors.[10,31]

- Skin care products should be applied daily (preferably several times daily) to hydrate the skin, prevent evaporation and allow autorepair through reconstruction of new lipids. The addition of carbamide and/or lactic acid helps to bind water, but in higher concentrations may irritate the skin.[31] Soaps with minimum defatting activity and a neutral pH are preferred.[9]
- Anti-inflammatory treatment. Topical glucocorticoids are used to control acute exacerbation of the eczema. Once control of the eczematous areas is achieved with a daily regimen of topical glucocorticoids, long-term control can be maintained with twice-weekly applications to regions that have healed but are prone to develop eczema. Side-effects of topical glucocorticoids are directly related to the potency and the length of use. Therefore high-potency agents should be used for very short periods of time and never in the face or intertriginous areas.[9]

  Tacrolimus and pimecrolimus (calcineurin inhibitors) are anti-inflammatory agents that inhibit production of Th1 and Th2 cytokines and the mediator release from mast cells and basophiles. Topical calcineurin inhibitors can be advantageous over topical corticosteroids when the response to topical corticosteroids is insufficient, in patients with steroid phobia, and in treatment of the face. Increased blood concentrations have generally not been reported in patients with moderate to severe AE. However, the potential risk of systemic absorption must be considered in children with extensive skin disease because of their high ratio of body surface area to weight. The drugs need to be carefully monitored to rule out the possibility that skin cancers and viral skin infections will appear when used long-term.[9]
- Infections. The colonization with staphylococcus aureus might lead to secondary infected eczema, which is most often exudative. Supplementary local treatment may be given with a solution of chlorhexidine (0.5-1.0 mg/ml) or potassium permanganate as a poultrice for a few minutes or as a bath ($KMnO_4$ 1-5 ml/liter water). $KMnO_4$ treatment dries out the skin and should be given once daily for a few days until the eczema is no longer exudative. Topical antibiotic products (such as mupirocine or fucidic acid) should be used externally for short periods only due to the risk of developing resistance and contact allergies. With pronounced infection oral antibiotics, such as a penicillinase-resistant penicillin or a cephalosporin, should be used.[9,31]

Eczema Herperticum is a rarely occurring, but serious condition involving the spreading of a cutaneous herpes simplex infection in the eczema. It should be treated with oral anti-herpes medication.[31]
Fungal infections (most often pityrosporum ovale) in the eczema should be treated with topical antifungal agents.[31]

- The itch is managed with topical anti-inflammatory agents and skin care. In children with AE and concomitant urticaria, oral antihistamines might be useful.[9] Irritants, such as dust, woolen fibers, water and soap should be avoided. Stress and sweating can worsen the itch, as can cold climate and high indoor temperatures.[31]

Gastrointestinal inflammation, eczema and nitric oxide

Altered intestinal permeability with increased absorption of large molecules, but with normal uptake of low weight molecules, has been reported both in eczema and food allergy, indicating inflammation of the intestinal mucosa.[32] Change in permeability, with altered absorption of PEG 400 and PEG 1000, has also been demonstrated after cow's milk challenge in milk allergic children. Modulation by pre-treatment with sodium cromoglycate showed that an anti-inflammatory drug reversed the reaction and its effect on the uptake.[33] Moreover increased gut permeability has been demonstrated in asthmatic children, suggesting that the entire mucosal system may be affected in allergic disease.[34]

Nitric oxide (NO) is a multipotent intracellular messenger modulating various physiological processes, including blood vessel dilatation and immune function, and can be produced by almost all mammalian cells. NO reacts rapidly with oxygen, yielding nitrite and nitrate, which are stable and can be measured in body fluids. NO is formed from L-arginine by NO synthase isoforms (NOS). There are two Ca^{2+} dependent constitutive forms (cNOS), eNOS and nNOS, and one Ca^{2+} independent inducible form, iNOS. The constitutive form, which produces low amounts of NO, has generally been associated with the regulating of the homeostatic function, whereas iNOS, which produces large amounts of NO and is induced in various cells by inflammatory stimuli, such as endotoxins and different cytokines, has been associated with severe tissue damage. However, iNOS has also been shown to have multiple positive biological effects. It is essential for normal healing of the skin and intestinal mucosa, kills certain bacteria, may be important in regulating T cell proliferation and the differentiation Th1 versus Th2, and may regulate leukocyte recruitment. Because all this may counter the effect of the toxic metabolites also produced by iNOS, it would be too simplistic to regard iNOS as only harmful. As there may be as many as 15 different types of cells that can express iNOS, it would also be inappropriate to assume that iNOS functions the same way in each cell. The fact that NO can produce toxic metabolites applies to a subset of cells, including oxidant-producing cells such as neutrophils, which, unlike epithelial cells, can produce large amounts of peroxynitrate. Significant quantities of eNOS and nNOS are found in the digestive tract under normal conditions, as are small amounts of iNOS. Inhibition of NO has been shown to cause the features of intestinal inflammation, including neutrophil recruitment, increased

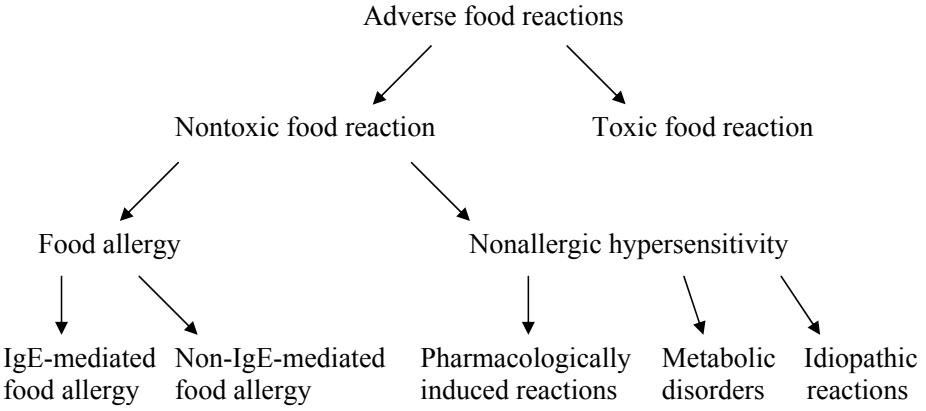
oxidative stress, mast cell degranulation, and increased microvascular and epithelial permeability.[35]

Atopy/allergy is characterized by a dysregulation of the pro-inflammatory and anti-inflammatory systems (Th1 versus Th2 cytokine balance). An inflammatory reaction, as in eczema, is thought to result in an activation of the stress system, which induces a Th1/Th2 shift, to provide protection from systemic "overshooting" with Th1-induced proinflammatory cytokines and elevated levels of toxic NO products.[36] However, the inhibition of NO may cause increased intestinal inflammation with mast cell degranulation and increased permeability, and could reduce the possible positive effects of NO, which may be important for skin and intestinal mucosa healing.[35,36]

When the Th1 response dominates, IFN- γ secreted by Th1 cells upregulates iNOS and increases NO, which in a feedback mechanism inhibits proliferation of both Th1 and Th2 cells. This arrest has been shown in airway mucosa to depend on NO because T cell proliferation is completely restored after in vitro blocking of iNOS. Variations in the efficiency of this feedback loop provide a plausible explanation of why only a subset of atopics progress to expression of relevant levels of inflammation.[37]

FOOD ALLERGY

Adverse food reactions are defined as any aberrant reaction after ingestion of a food or a food additive. An adverse food reaction may be the result of a toxic or nontoxic food reaction. *Toxic food reaction* can occur in anyone, provided a sufficient dose is ingested, whereas *nontoxic food reactions* depend on individual susceptibilities and may be the result of immune mechanisms, *food allergy*, or *nonallergic food hypersensitivity*, as in food intolerance.[2,38] *IgE-mediated food allergy* has been most clearly delineated, but non-IgE-mediated immune reactions, especially in the gastrointestinal tract, are being increasingly recognized. Food intolerance probably accounts for the majority of adverse food reactions and may be caused by pharmacological properties of the food (e.g. tyramine, caffeine), or unique susceptibilities of the host, such as metabolic disorders (e.g., lactase deficiency) or idiopathic responses.[38] Food aversions might also mimic adverse food reactions, but are not reproducible when the child ingests the food in a blinded fashion.[39]



Food allergy mechanisms

It has been suggested that the development and the rather high incidence of food allergy in infancy is due to an incomplete mucosal barrier, increased gut permeability to large molecules, and immaturity of local and systemic immunologic responses.[23] Despite the complex mucosal barrier with both physiochemical factors, such as tight junctions, mucus layer, enzymes and bile salts, and cellular factors, such as natural killer cells, macrophages, polymorphonuclear leucocytes, lymphocytes, sIgA and cytokines, preventing the penetration of foreign antigens, about 2% of ingested food antigens are absorbed and transported throughout the body in an immunologically intact form, even through the normal mature gut. Developmental immaturity of various components of the gut barrier reduces the efficiency of the infant mucosal barrier. For instance, enzymatic activity is suboptimal in the newborn period, and the IgA system is not fully mature until four years of age.[39]
In general, the normal immunological response to orally introduced food antigens involves induction of tolerance, leading to unresponsiveness upon further exposure to the antigen.[40] Sensitization to food allergens can be considered a failure of the normal induction of oral tolerance, which is mainly dependent on activation of antigen-specific regulatory T cells that produce the immunosuppressive cytokines TGF-β and IL-10. Allergic sensitization seems to occur preferentially following stimulation with low rather than high amounts of antigens. Sensitization to food allergens is most pronounced during infancy, when mechanisms of tolerance are less well developed.
In non-atopic children the development of IgE antibodies to food allergens is a transient phenomenon, but in atopic infants, having an imbalance of the Th1/Th2 system facilitating the production of IL-4 and 13, the IgE antibody production persists and gives rise to allergic symptoms. As there is a deficiency of IFN-γ production in atopic infants, the physiological IgE response to food antigens that occurs in early infancy will not be adequately downregulated.[40]

Development of oral tolerance is also dependent on the intestinal microflora. LPS (lipopolysaccharide from *E.coli*) is a potent inducer of the immunosuppressive cytokine IL-10. Therefore a lack of stimulation of the immune system by intestinal bacteria containing LPS may favor the development of allergic sensitization. Lactobacilli and gram-positive cocci have a special ability to induce the formation of IL-12, which is a key cytokine of Th1 immunity. According to the Th1/Th2 immunity concept, this may counteract allergic sensitization, which is Th2-dependent.[40]

Failing maturation of the immune system because of abnormal production of prostaglandins and hydrogen peroxide might also influence allergic sensitization, increasing the Th2/Th1 (IL-4/IFN-γ) ratio. The abnormal production of prostaglandins may be caused by high omega-6/omega-3 fatty acid ratio, and increased levels of hydrogen peroxide by low contents of antioxidants in the food. The increase in the prevalence of food allergy in the western world has occurred in parallel with changed food habits regarding omega-6 and omega-3 fatty acids as well as antioxidants.[40]

Although allergic sensitization can be assumed to be induced mainly via orally introduced food, it also seems possible that sensitization to foods may take place via the skin e.g. around the mouth of infants, and that this sensitization is normally counteracted by tolerance-inducing mechanisms of the gut.[40]
The clinical impression has been that strict avoidance increases the chance of outgrowing the food allergy and may even hasten the process. However, very few data support this notion and in practice some children rapidly outgrow their food allergies without strict avoidance, whereas others fail to lose their allergies even with the most stringent diet.[12,41]

Clinical manifestations in food allergy

The IgE-mediated reactions occur when food-specific IgE antibodies residing on mast cells and basophils come in contact with and bind circulating food allergens and activate the cells to release potent mediators and cytokines.[39]
Early indications of a reaction in young infants can include subtle signs such as moving the tongue in the mouth to rub the itchy palate, or ear pulling due to referred pruritus. They may also become suddenly quiet or assume a fetal position as a prodrome to more objective symptoms.[42] Gastrointestinal allergic symptoms present as acute nausea, abdominal pain and vomiting, and generally occur with allergic manifestations in other target organs. The cutaneous reactions in IgE-mediated food allergy are eczema, erythema, urticaria and angioedema. In the respiratory tract the food allergy presents as allergic rhinoconjunctivitis and asthma.[39]
The IgE-mediated food allergy can also cause a generalized anaphylaxis. The symptoms of anaphylaxis are cutaneous (urticaria and angioedema), respiratory (asthma), gastrointestinal and cardiovascular (hypotension and anoxemia).[17] Food-associated exercise-induced anaphylaxis is a special form of anaphylaxis that occurs only when the patient exercises within two to four hours of ingesting a food.[39]

In milk allergy most infants develop symptoms during the first months of age, often within one week after introduction of formula based on cow's milk. Onset after one year of age is extremely rare. The majority of children have two or more symptoms, with symptoms from two or more organ systems. In exclusively breastfed infants, severe AE is a predominant symptom.[43] Symptoms may occur within a few minutes to two hours after milk exposure (immediate reactions) or after two hours (late reactions), and in some cases after several days. Most often, the late reactions are non IgE-mediated.[15,43]
Most children outgrow their allergy to milk, but different results in tolerance achievement in IgE-mediated milk allergy have been shown, including 76% by age 3[44], 56% by age 4[45], 78% by age 6[45], 38% by age 7[46], and 57 % by age 8.[47] The varying results reflect differences in study design, diagnostic criteria, age at entry into the studies, and the duration of the follow-up period.[43] However, the prospective population-based study by Høst et Halken, showed that already at the age of 3, 76 % of children with IgE-mediated food allergy, proven by strict elimination/challenge procedures, had achieved tolerance and when also non-IgE mediated allergy were

included, 87% of the children could tolerate milk.[44] In children with egg allergy, clinical tolerance was achieved in 30-44% by school age.[48,49]
Development of allergy to aeroallergens occurs mostly in infants with high levels of specific IgE to milk and egg and has been shown to be as high as 80% at schoolage.[9,43] In these infants, inhalant allergy typically develops to substances to which the children have been exposed from early infancy, i.e. house-dust mites, cat and dog.[43] They have also an increased risk of persisting adverse reactions to other foods.[43]

Food allergy and its effect on nutritional intake, growth and quality of life

Any diet during infancy or early childhood represents a major intervention for the child.[50] At present however, avoidance of the food allergen source by following an elimination diet is the only treatment of food allergy. This is a challenging task because food manufactures use many of the common food allergens in their products. Children with milk allergy or multiple food allergies are shown to be at greater risk of impaired nutrient intake and growth. Also eczema has been implicated as a risk factor for delayed growth of children due to increased nutrient requirement associated with the inflamed and chronic state of the eczema.[51] In families with food-allergic children the activities of daily life are potentially impacted by concerns about cross-contamination of foodstuffs in various settings, and the risk of accidental exposures in school, childcare, and social activities.[52] Up to a third of households have altered their eating habits in the belief that at least one family member is allergic to a food.[13]
Accurately identifying children with a clinically relevant food allergy will help to prescribe specific diets on a scientific basis and avoid dietary limitations which may be unnecessary or even harmful for the children.[15,53] Because the diagnosis of food allergy is clearly life-altering, it is crucial that it is based on solid evidence.[52]

Diagnosing food allergy

As adverse reactions to foods can have many different origins, diagnosing food allergy can be difficult at times. The most common way of beginning to search for diagnosis is to penetrate the history:

- What are the suspected foods?
- How much time has passed between the ingestion and the onset of symptoms?
- Is the reaction frequent and reproducible?
- What symptoms did the reaction elicit?
- Is there an atopic heredity?
- What are the environmental factors?

Standardized questionnaires may be useful tools.[11] However, no symptoms are pathognomonic for food allergy, and no single laboratory test is diagnostic.[23] A history of an adverse food reaction or even evidence of sensitization does not necessarily

mean that the child will exhibit a clinical reaction on exposure to that food.[12] Sensitivity to parental history has been shown to be 48%, and specificity 72%.[15]

As well as the clinical history, testing for food allergy includes the following investigations:[11]

- Skin prick test (SPT)
- Total and specific IgE in serum
- Food challenge
- Atopy patch test (APT)

Proof of a positive SPT or measurements of specific IgE antibodies may represent a short-term reaction in infancy with no correlation with oral clinical allergy, and these tests can also remain positive long after a child who has been food allergic has achieved clinical tolerance.[12,15,54] In contrast children younger than 2 or 3 years are more likely than older children to have a negative SPT and a positive challenge.[55]
In the context of a poor clinical history, a 3 mm SPT weal does not support a diagnosis of allergy. At the other hand, a child with a convincing history of food allergy and a negative SPT should undergo food challenge.[56] However, in the case of an acute IgE-mediated reaction, such as urticaria or anaphylaxis after eating a particular food, a positive SPT or specific IgE in sera would be confirmatory. At the other hand, in chronic disorders, such as eczema and asthma, or with delayed gastrointestinal reactions, it can be more difficult to pinpoint causal foods. Confirming or refuting the diagnosis of food allergy in these chronic disorders usually requires oral challenges.[42]

If cell-mediated reactions to food are suspected, particularly in AE with negative tests for IgE-mediated allergy, the evaluation can be extended with the use of APT for identifying these late food reactions.[57] However, at this time there is no agreement with respect to standardized reagents, or methods of application or interpretation. In addition, as nonspecific irritation is a common finding in APT, the interpretation requires skill.[39]

Children diagnosed with food allergy and prescribed an elimination diet should be re-evaluated at regular intervals, most often annually, to determine whether the allergy has been outgrown.[12] As in the procedure for diagnosis, the re-evaluation process may include SPT, IgE analyses in sera, and/or oral food challenges.

Skin prick tests and specific IgE in sera

SPT and specific IgE in sera are sensitive indicators of food-specific IgE antibodies but poor predictors of clinical reactivity. Consequently, the tests may be considered an excellent means of ruling out IgE-mediated food allergies, but are only suggestive of the presence of clinical food allergy.[58-60] Approximately 85% of children with eczema have elevated serum IgE, and about 85% of these have specific IgE antibodies to food and inhalant allergens.[19]

Skin prick test

The SPT method, used extensively in children, has several advantages. Reactions can be read after 15 min, making it quick. Parents can see the reaction of the positive test result on the skin, giving the method pedagogical advantages. The method is also relative inexpensive compared with serologic methods for allergy investigation.

The SPT to foods method

The SPT should always be performed as a standardized method, and the investigator should be well trained in the procedure of both the test performance and interpretation of the results. The proteins in commercial food extracts are prone to degradation, and different lots of food antigen extracts from the same company have been found to vary in potency by a hundredfold. Therefore fresh or fresh-frozen foods (skimmed milk and egg white, preferably from several eggs) and the prick-prick manner of testing are recommended.[42,61,62] The lancet used should be a metallic, with a 1 mm tip (ALK® or Dome®).[62]

Recommendations for the SPT performance: [62]

- The SPT is performed on the volar aspect of the forearm.
- The skin is marked before the test with a ballpoint pen for the allergens to be tested.
- The tip of the lancet is dipped into the food and then pressed at right angles against the skin surface for one second (count 1001). Use the volar aspect of the finger tip.
- The intervals between the pricks should be 2 cm.
- As a negative control, prick the skin with a clean lancet.
- As a positive control, prick the skin with histamine dihydrochloride 10 mg/ml.
- The reaction is read after 15 minutes.
- The test is considered positive when the mean diameter (= half of the sum of the largest diameter and its perpendicular) of the wheal is $\geq$ 3 mm greater than the negative control.

Oral antihistamines may significantly suppress the skin response and should be withdrawn for at least 2 days. High-potency topical steroids to the test area should be seponated for 2-3 weeks prior to SPT.[63]

Risks

Skin prick testing is regarded as a safe procedure. The method has replaced the former use of intradermal skin tests, in which anaphylactic reactions were not unusual, and even deaths were reported .[64] In 1989, Turkeltaub et al reported minor adverse reactions in only 0.04 % in SPT-tested patients, age >6 years, and Lin et al found the frequency to be 0.02% in a study performed in 1993.[65,66] Nevertheless, the SPT can

induce systemic reactions in highly sensitive patients, even if such reactions are rare. Two cases of anaphylactic reactions were reported in 1995 by Novembre et al after skin prick tests using fresh foods as the test substance.[67] Furthermore, Valayasevi et al, in their study of over 18 000 patients, reported six systemic reactions, all in patients with asthma, when skin testing was performed.[68]
The risk of severe generalized reactions may increase because investigators have lost their former respect for skin testing as SPT has replaced the more hazardous intradermal testing.[69] Therefore it is important to always make sure that the SPT is performed in a standardized manner and that necessary precautions are taken.

Precautions when performing SPT [67,70]

- Do not test when there are ongoing allergic reactions.
- In children with a history of anaphylaxis, first apply the wet food on the intact skin for some minutes before performing the prick-prick test.
- Even if the amount of allergen introduced with SPT is very small, the possibility of a summation of the reactions in the case of multiple positive skin tests should be kept in mind, and in highly sensitive children only a few tests should be performed at the same session.
- Even with a low risk of generalized reaction, SPT should be performed with both a nurse and a doctor present.
- Rescue medication i.e. epinephrine, should be prepared and ready to be used if a generalized reaction should occur.

Outcome

Parameters for defining the diagnostic properties of SPT are sensitivity, specificity, positive and negative predictive values (PPV and NPV), and positive and negative predictive accuracy (PPA and NPA), Appendix 1 .
EAACI position papers recommend $\geq$ 3 mm diameter of the wheal as cut-off value for positive result, since SPT <3 mm is not predictive of clinical food allergy.[63]

Values when using cut-off > 3 mm in SPT to milk [60,61,71]

- Sensitivity 73-96%
- Specificity 51-90%
- PPA 40-78%
- NPA 83-99%

Values when using cut-off > 3 mm in SPT to egg [60,61,71,72]

- Sensitivity 87-98%
- Specificity 53-71%
- PPA 40-85%
- NPA 85-96%

Cutaneous reactivity varies with age and the clinical context. The wheal size in positive SPT results is smaller in young infants.[73] The PPA at cut-off level >3 mm in

SPT to egg has been shown to differ between 40% in children > 3 years and 80% in children < 3 years.[72] There is also a considerable variation in SPT outcome between different commercial food extracts, resulting in a variation of 0-79% in PPA.[61]
Practising allergists would want to know the SPT diameter to which all patients will react on challenge (100% specificity) in their high-risk populations to decide whether or not to undertake an (expensive and potentially life-threatening) challenge, whilst avoiding unnecessary diet restrictions. In fact cut-off values that will provide both high positive and high negative predictive values would be the most useful in the clinical setting, because this would indicate which patients are likely to have symptoms in response to a certain food and which are probably non-reactive.[60] If applying cut-off values of > 8 mm for milk and > 7 mm for egg (in infants < 2 years of age, > 6mm and >5 mm, respectively), the specificity and PPV will reach 100%, but the values for sensitivity and NPV will then decline substantially to < 18% and < 37%, respectively.[74,75]

In conclusion, SPT when using a cut-off limit of ≥3 mm wheal diameter has high sensitivity and has shown to be highly useful in excluding IgE-mediated food allergy, whereas the specificity is too low to be of value in identifying clinically relevant allergy. Positive predictive accuracies with this cut-off limit are generally poor, but negative predictive accuracies are good.[61] The outcome of SPT has been demonstrated to vary considerably with age, season, gender, clinical context, and method and extracts used. Therefore different cut-off values are likely to be required for different subpopulations of children, and the results of different studies should be compared with great caution.[56]

Specific IgE in sera

RAST and similar qualitative in vitro assays provide suggestive evidence of IgE-mediated food allergy, but these assays are giving way to quantitative measurements of food-specific IgE antibodies (e.g. Pharmacia CAP system specific IgE, FEIA®), which have been shown to be more predictive of symptomatic IgE-mediated food allergy.[39] Determination of food-specific IgE/total IgE gives similar results to the determination of food-specific IgE alone, making it an unnecessary effort to calculate the ratio.[76] The method holds excellent sensitivity and negative predictive accuracy, but poor specificity and positive predictive accuracy.[60]
The UniCAP® 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, allowing for quantitative assessment of allergen-specific IgE antibodies in kilo units of allergen-specific IgE per liter (kU_A/L).[60]

Principle of the UniCAP® Specific IgE assay [77]

The allergen covalently coupled to ImmunoCAP reacts with the specific IgE in the patient serum specimen. After washing away non-specific IgE, enzyme-labeled

antibodies against IgE are added to form a complex. After incubation, unbound enzyme-anti-IgE is washed away, and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the response value, the more specific IgE in the specimen. To evaluate the test results, the response for the patient samples is transformed to concentrations with the use of a calibration curve.

Values		*Class*	*Allergen-specific IgE antibody*
<0.35	kU_A/L	class 0	absent or undetectable
0.35-0.7	kU_A/L	class 1	low
0.7-3.5	kU_A/L	class 2	moderate
3.5-17.5	kU_A/L	class 3	high
>17.5	kU_A/L	class≥4	very high

Outcome

Specific IgE in sera is a measure of 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.[58]

Values when using cut-off value >0.35 kU_A/L in specific IgE to milk [15,60,78]

- Sensitivity 83-100%
- Specificity 30-53%
- PPV/PPA 63/57%
- NPV/NPA 71/100%

Values when using cut-off value >0.35 kU_A/L in specific IgE to egg [15,60,78]

- Sensitivity 95-98%
- Specificity 38-51%
- PPV/PPA 79/84%
- NPV/NPA 75/88%

Different studies have attempted at define high positive predictive decision points, resulting in PPA >95% for egg at >6 kU_A/L and milk at >32 kU_A/L,[60] and for egg at >7 kU_A/L and milk at >15 kU_A/L.[79] The overall specificity at these levels is 90-100%, but the sensitivity and NPA fall to 34-64% and 38-53%.[60,79] To reach NPA > 95% the decision point for milk has to be set at 0.8 kU_A/L, and for NPA > 90% for egg at 0.6 kU_A/L.[60] It should be observed, however, that with a specific IgE level <0.35 kU_A/L an allergic reaction might still occur,[39] and there is no correlation between the level of food-specific IgE and the severity of the allergic reaction.[60] Sampson suggests rechallenging children when their food-specific IgE levels decrease to one fourth the diagnostic decision point, unless they have experienced a recent allergic reaction.[80] Children with symptoms of food allergy and lower levels of food-specific IgE antibodies are found to more likely "outgrow" their reactivity at a young age, whereas children with high levels of IgE antibodies (i.e. three to four times the 95% predictive diagnostic value) are unlikely to lose their reactivity for many years (i.e. > 5 years).[60]

Since the variability in the specific IgE level predicting clinical egg and milk allergy has varied from 0.35 kU_A/L to 32 kU_A/L or even greater; data in one population cannot readily be transferred to other clinical settings.[56,60,79,81]

Future developments in the use of specific IgE

Recent advances in technology have enabled investigators to map allergenic epitopes of many major food allergens and determine specifically where individual patient's IgE antibodies bind to these proteins. Both conformational and sequential epitopes might be responsible for the allergic reactions, but individuals who possess IgE antibodies to sequential epitopes react to the food in any form, whereas those with IgE antibodies to conformational epitopes appear to tolerate small amounts of food after heating or partial hydrolysis because the tertiary structure of the protein is altered and the conformational epitopes are destroyed. In addition, those with egg and milk allergy with IgE antibodies directed at sequential epitopes tend to have persistent allergy, whereas those with IgE antibodies primarily to conformational epitopes tend to develop clinical tolerance. Patients with IgE antibodies binding to many epitopes tend to have more severe allergic reactions compared with those who have IgE antibodies binding to relatively few epitopes. New technology under development (protein and peptide microarrays) might some day enable physicians to screen patients to a number of foods and tell whether they will react to a specific food, identify potential cross-reactivities on the basis of homologous epitopes, and predict how severe their reaction might be and whether they are likely to outgrow their allergy.[39]

Oral food challenge – standardized open and double-blind placebo controlled

It has been suggested that the best initial approach to screening for food allergy is the use of open or single blind food challenge directed by SPT,[82] whereas double-blind placebo controlled (DBPCFC) is the appropriate and only reliable method of evaluating and confirming a suspected adverse food reaction and represents the "gold standard" for diagnosing food allergy.[15,42,81] A positive outcome of DBPCFC confirms the diagnosis of a true allergic reaction to a food and allows an estimate of the dose required to induce symptoms.[54]

Although several studies have examined DBPCFC in children with suspected food allergy, little is still known about the various patterns of clinical reactions during the challenge (time-course, distribution of early and/or late reactions, organ-specificity of allergens, association between titration dose and clinical reaction, differences due to the children's age) and the value of specific IgE measurements.[15] A great variety of challenge regimes has been used by clinicians and researchers, all with different suggestions, for instance concerning the amount of food to administer at the challenge procedure. These include: a 'relevant amount of food'[14]; 'a total amount approximating an age-related average daily intake'[53,55]; and 'approximate a serving of food'.[54]
Different protocols for the DBPCFC have been used over time, but no standardized procedure has been agreed upon, making it difficult to compare results between

different centers and different subpopulations.[83] Recently both advice for standardization of the method and a protocol for low-dose food challenge with smaller doses than in conventional food challenge have been published.[83,84] In the low-dose protocol the aim of each challenge was to elicit clinical symptoms in highly sensitive patients to find the threshold levels that would protect the vast majority of allergic consumers. However, this model is not suitable in young children outgrowing their food allergy as there is a desire to show parents that their child can eat small amounts of the food without incurring an allergic reaction.
The successful administration of oral food challenge to young children requires a great deal of preparation, ingenuity, and often patience.[42] Developing new recipes takes a long time, and one has to make sure that correct blinding is given, e.g. by a dietician.[53,85] The vehicle must allow for a truly blind challenge, masking the smell, flavor and texture of the food.[59,85]
Assessment of possible differences between active samples and placebos should be evaluated by standard procedures as in the triangle test, where a taste panel compares three samples to detect differences regarding taste, texture, smell, etc.[83] Children may also reject a food because it has a strange taste, and their resistance can even lead to vomiting for emotional reasons.[55] Having additional challenge vehicles, for example liquid and solid forms of the substance, readily at hand may prevent delays.[42]

In which children should the food challenge be performed?

Algorithms mainly based on measurements of specific IgE levels and SPT outcome, have been presented as tools for deciding when and how food challenges in childhood should be performed.[86] Such algorithms should, however, be used with great caution especially in infants < 2 years of age. Following them may also involve a risk that inexperienced doctors will consider performing these tests. Food challenges in infants and young children with significant food allergy should always be evaluated and performed by an experienced paediatrician.[41]

The oral food challenge should be performed: [83]

In children with a history of adverse reaction to a 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, mainly to egg and milk.
- For scientific reasons in clinical trials.

In children without specific history of adverse reaction to a 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 a sensitization to a food is diagnosed and tolerance is not known, e.g. sensitization to cross-reactive foods.

The oral food challenge should not be performed: [83]

- 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 allergy).
- In children with chronic atopic disease such as asthma and AE if the disease activity is not stable.
- In children taking medication which may enhance, mask, delay or prevent the evaluation of a reaction or interfere with treatment of a reaction (e.g. oral antihistamines, oral steroids).

Recommendations for oral food challenge;

- Ensure that clinical monitoring is standardized (e.g. symptom-scores); optionally, monitoring by mediator measurements from effector cells is recommended.[53]
- Suspected foods should be eliminated at least 7 to 14 days before challenge.[39]
- A challenge is considered positive for IgE-mediated food allergy when objective symptoms occur within 2 hours of the oral challenge.[54,61,71] Clinical reactions after 2 h are defined as late reactions.[15] The time should be counted from highest dosage, not from first dosage.[53]
- Performing two challenges on the same day, one with the suspected food and the other with the placebo, will not allow for an evaluation of late reactions.[55]
- Natural food should be offered in the way the patient would normally eat it.[14,53,55] Freeze-drying can alter the food's allergenic potential, as can cooking. Therefore minimize the processing of foods.[55,85]
- Capsules are the best method for blinding, but they are not well tolerated by children, especially the younger ones and the allergen is usually not native and does not test oral tolerance.[54,55,59]
- The starting dose should be half the minimum quantity estimated by patients to have produced the symptoms (range 25-500 mg).[55]
- The increment may either be a doubling of the dose until the top dose has been reached or the child reacts, or an increment using logarithmic mean (i.e. 1, 3, 10, 30, 100 etc).[83]
- The top dose should normally be the normal daily intake in a serving of the food in question, adjusted for the age of the patient.[83]
- The dose can be doubled in intervals slightly longer than the one reported needed for the onset of symptoms, usually 15-30 min (30-60 min if capsules are used).[55,83]
- The concentration of the suspected agent hidden in the food should be as high as possible without being detectable.[85]
- The placebo should be identical in flavor, colour and consistency to the one containing the allergen.[55,85]

Outcome

Even with DBPCFC, it is sometimes difficult to assess the outcome of the challenge, especially in infants and young children. Target organs affected appear unpredictable and may vary for different foods.[14] This especially holds true for late phase reactions, which are mostly eczematous reactions and to some extent gastro-intestinal. Controversy exists, concerning whether there are isolated late reactions or if they only occur in combination with early reactions.[53] Breastfeeding during provocation may also influence the outcome of the challenge.[53] The average false-positive rate for the DBPCFC has been reported to be 0.7% and the false-negative rate to be 3.2%.[13] Previous studies have shown that symptoms will occur within 2 hours of ingesting the food allergens.[60,61] In challenges with positive outcome 49% (egg) and 55% (milk) of the infants will react at the first dose, and 11% of the reactions that occur at the first dose will be severe.[14]

Open challenges are good for defining non-reactors but are more likely than blinded challenge to give false positive results.[55] Performing the challenges blinded reduce errors to a minimum not only in research, but also in daily practice, and in some studies confirm only 50% of the positive open challenges.[55] Even in studies of highly selected patients, only 60% or fewer are found to have reproducible reactions in DBPCFC.[59] When the AE is severe, the prevalence has been shown to be higher, approximately 65%.[54]
In young children (< 2-3 years of age) the open challenge is most often sufficient, whereas in older children DBPCFC might be necessary at times to rule out positive reactions due to psychological mechanisms.[11,83] If reactions due to Münchhausen by proxy syndrome are suspected the DBPCFC is also helpful.[11]

After a negative blind challenge, either an open challenge should be performed or the parents should be instructed to add food to the diet in small but increasing amounts for several days.[72] Occasionally an open challenge, following a negative blind challenge, will be positive. False negative challenges are mainly due to dose-response mechanisms. This is particularly evident when patients are acquiring a tolerance to a given food. Other causes could arise from different allergenicity of the food, the loss of allergenic potential due to cooking or processing, or psychological factors.[55,59] There are reports of reactions to raw egg following negative challenge with cooked egg.[13] Therefore parents should be instructed that the negative challenge with processed egg does not exclude reactions to raw egg.
Once the food is tolerated, it can be eaten as often as the patient desires, and in usual portions.[72]

Risks

Performing oral food challenges is not without risk. Therefore challenges should never be done at home if there is even a remote risk of a severe reaction occurring.[59,82] The size of the SPT wheal and specific IgE levels in sera are not predictive of how severe the reaction will be when the outcome of the food challenge is positive.[60,75]

Precautions when performing food challenge: [83]

- Even with a low risk of generalized reaction, SPT should be performed with both a nurse and a doctor present and in a hospital setting.
- Rescue medication (i.e. epinephrine for injection and oxygen, β2-agonists for inhalation, steroids for injection), should be prepared and ready to be used if a generalized reaction should occur.
- Intravenous access should be available before initiation of the challenge as a general rule and always if a severe systemic reaction is expected.
- The child should stay for an observation period of a least 2 hours after the last dose administered.
- In cases where a severe reaction might be suspected, the challenge should be performed in settings with immediate access to intensive care units.

When to re-challenge?

Since children generally outgrow allergies to cow's milk and to eggs, re-evaluation after about one year would seem appropriate. When the concentration of egg-specific IgE is less than 1.5 kU_A/L or the milk-specific IgE is less than 5 kU_A/L and the mean wheal diameter is less than 7 mm, it has been suggested that one can proceed with the DBPCFC, followed by an open feeding.[13] In addition, a greater decrease in IgE levels in sera over a shorter period of time has been shown to be indicative of a greater likelihood of tolerance development.[88] As there is no laboratory parameter which can accurately predict when clinical tolerance is achieved, controlled oral food challenges are necessary to prove that tolerance has developed.[88]

Treatment of food allergy

At present there is no effective therapy for IgE-mediated food allergy. Elimination of the offending food is the only way to avoid clinical food reactions. Children with food allergy and asthma or a previous severe reaction should be prescribed an auto-injector of epinephrine and be instructed how to use it in case a generalized reaction occurs. Antihistamines might partially relieve symptoms of oral allergy syndrome and IgE-mediated skin symptoms, but do not block systemic reactions.[39] Acute food allergy induced asthma should be treated with inhaled or systemic corticosteroids and inhaled β2-agonists. Topical corticosteroids are effective in treating AE, as is skin lubrication.[89]

A number of novel therapeutic modalities are at present being explored, such as injections of anti-IgE-antibodies, vaccination of plasmid DNA, the use of anti-allergy immunostimulatory sequences, cytokines and bacterial agents, immunotherapy with mutated proteins and peptides, and complementary medicine such as traditional Chinese herbs.[90] In the future these might add to the possibilities to treat and even prevent food allergy.

Appendix 1.

Statistics for the IgE tests (skin prick test and specific IgE in sera) and the food challenge [54,61]

Sensitivity and specificity are statistical measurements of how well a diagnostic test correctly identifies or confirms cases of disease.

Sensitivity (SPT) = Positive food challenges correctly identified by the IgE test (TP)/ Total number of positive food challenges (TP+FN)

Specificity (SPT) = Negative food challenges correctly identified by the IgE test (TN)/ Total number of negative food challenges (TN+FP)

Predictive values indicate how likely an individual is to have a disorder given the results of the diagnostic test.

Pos predictive value (PPV) = Positive food challenges correctly identified by the IgE test (TP)/ Total number of positive SPT (TP+FP)

Neg predictive value (NPV) = Negative food challenges correctly identified by the IgE test (TN)/ Total number of negative SPT (TN+FN)

The sensitivity, specificity, and predictive values (efficiency) of a diagnostic test provide information about its ability to identify a known condition and thus provide some insight into the utility of the test in clinical practise.

However, in practise the physician is more interested in knowing how likely it is that the patient has the disorder if the diagnostic test result is positive, Positive predictive accuracy (PPA), or how likely it is that the patient does not have the disorder if the test result is negative, Negative predictive accuracy (NPA). The predictive accuracies are much more difficult to establish than the sensitivity and specificity because they are heavily impacted by the prevalence of the disorder in the population being studied.
The higher the prevalence in the study group, the better the positive predictive accuracy will appear. The lower the prevalence, the better the negative predictive accuracy will appear.

$PPA = sP / sP + (1-f)(1-P)$ $NPA = f(1-P) / f(1-P) + (1-s)P$

s = sensitivity f = specificity
P = prevalence of the condition in the population under study

Consequently, if the prevalence of food allergy in the patient population to be studied is not the same as that found in the population that was used to establish rates, a correction must be made to determine the expected accuracy of the test. Without such a correction, one might erroneously conclude that the PPV of a test is sufficient to predict reactivity.

The thesis

Assessing eczema and food allergy in young children

AIMS OF THESIS

To study the natural cause of eczema and food allergy in young atopic children, and evaluate and further develop the methods used for investigation.

I To examine detailed case studies of generalized allergic reactions in connection with skin prick testing in order to identify possible risk factors and thereby increase the safety of the test procedure.

II To investigate the necessity of performing skin prick tests in duplicate.

III To develop recipes and a protocol for low-dose oral food challenge to milk and egg to be used for standardized open and double-blind placebo-controlled food challenge (DBPCFC) in young children outgrowing their food allergy so as to facilitate early re-/introduction of small amounts of milk and egg into the diet.

IV To evaluate the urinary levels of nitric oxide (NO) breakdown products in children with eczema, with and without sensitization to food allergens, and to assess the effect of eczema treatment on clinical symptoms and the NO levels.

V To evaluate the atopic march in children with eczema, with and without sensitization to foods, from referral at under 2 years of age until follow-up at 4½ years of age.

MATERIAL AND METHODS

STUDY I

A retrospective assessment of medical records of six infants, all of whom developed generalized allergic reaction in connection with skin prick tests performed between 1996 and 1998. We studied in detail the history of the cases observed to see whether the children shared any common features that could be regarded as risk factors.
Information about the total number of skin prick tests performed during the period and distribution according to age was collected from the computerized database at the clinic. The tests were performed in children 0 to 19 years of age at the Pediatric Clinic at the University Hospital in Linköping, Sweden.

Statistics
Confidence interval 95% for one proportion was calculated according to Altman.[92]

STUDY II

A retrospective analysis of all skin prick tests performed in duplicate at the Pediatric Clinic at the University Hospital in Linköping, Sweden, in 1997. Tests performed with the single prick method were excluded from the study. Duplicate skin prick testing has previously been recommended because of the risks of accidental negative tests.[91]
The skin tests were performed by the same two specially trained and experienced nurses and in a standardized manner.[62] To document the results, the wheals were outlined by a soft ballpoint pen. The markings were then transformed to the result chart by means of cellotape. The test results were kept in the medical record of each child. The wheals were measured, and the mean diameter (= half the sum of the largest diameter and its perpendicular) was calculated. We registered whether the outcome was two positive, two negative, or one positive and one negative test result. The skin prick test was considered positive when the mean diameter was at least 3 mm for foods, and at least 2 mm for inhalation allergens.[93] All the results were analyzed by the same investigator.

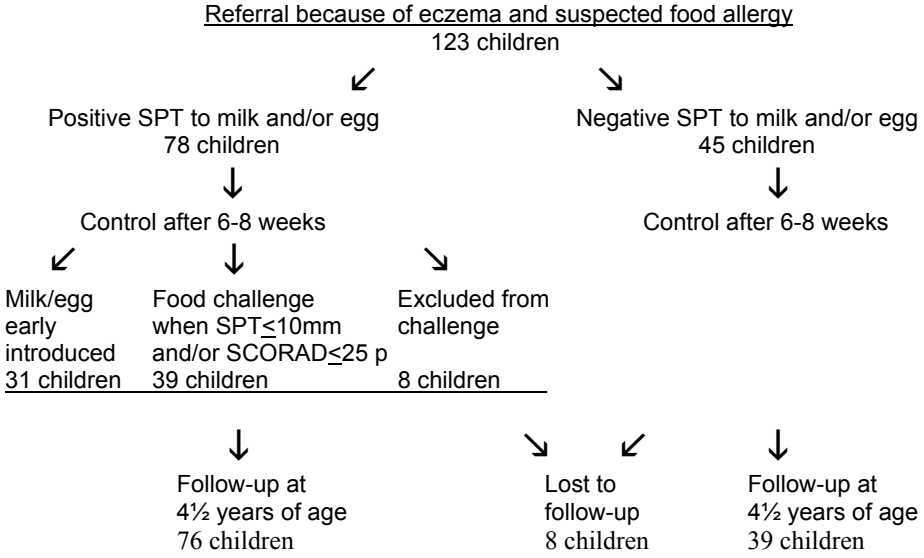
Statistics
Confidence interval 95% for one proportion was calculated according to Altman.[92]

STUDIES III-V, The study population

The entire study population consisted of 123 children (71 boys, 52 girls) under two years of age (median 6 months, range 1-23) on referral from primary-care physicians because of eczema and suspected food allergy. The children were recruited to the study over a two-year period, from June 1999 to September 2001, at the pediatric clinics in Linköping (n=53), Jönköping (n=44), Hudiksvall (n=12) and Norrköping (n=14), Sweden. All children fulfilling the inclusion criteria (referred at age < 2 years, eczema and suspected food allergy) were invited to participate in the study. In Linköping we analyzed the initial drop-outs; 53/109 (49%) agreed and 56/109 (51%) declined to participate. The reasons were language/communication problems in 21%, participating in other studies in 14%, complicating diseases in 5%, and not interested in taking part in the other cases.

At first visit we enquired about other atopic manifestations, family history, environmental factors, and the nutritional supply. The eczema was evaluated with the SCORAD instrument[30], and Hanifin-Rajka criteria were used to diagnose atopic eczema.[29] The children were tested for sensitization to milk and/or egg (the main food allergens in Swedish children of this age group) using the SPT with a cut-off limit of ≥3 mm and performed in a standardized manner as described previously (I). The SPT and the SCORAD assessment were performed by experienced allergy research nurses. Before the start of the study, the nurses practised scoring on children with eczema to reduce inter-observer variability. On positive SPT outcome, the parents were instructed by a dietician to eliminate the offending allergen, and if breastfeeding, the foods were also excluded from the mother's diet. For the first six months of life they were either exclusively breast-fed or received a documented hypoallergenic formula (Nutramigen®). The parents also received instructions and were given a practical demonstration of how to treat eczema and dry skin. Emollients for skin care were provided as well as anti-inflammatory treatment with topical glucocorticoids if needed. Blood samples were obtained, and total and specific IgE in serum were measured. Morning urinary samples were obtained at the first and second visits (after 6-8 weeks). The children were re-evaluated at regular intervals clinically, and with SPT and SCORAD. At the first visit 78 children tested positive for milk and/or egg. In 31 children the foods were re-introduced early either by the parents at home, who did not wait for a controlled challenge or did not comply with the elimination diet, or by open challenge after a short elimination period. These children were regarded as food sensitized but not food allergic. The remaining 47 milk and/or egg-allergic children, in whom early food challenge could not be performed due to severe eczema, SPT > 10 mm or recent allergic reactions on accidental exposure to the offending foods, were re-evaluated annually. Eight children were excluded from challenge because of SPT > 10 mm and/or severe generalized allergic reactions on accidental exposure to the foods within the previous six months. The remaining 39 children underwent oral food challenge when the SPT was ≤ 10 mm and SCORAD ≤25.

When the children were 4½ years of age, we conducted a follow-up in all the children to assess eczema and carried out SPT tests for milk, egg and aeroallergens. We enquired about other allergic manifestations and clinical tolerance to foods. A clinical examination was conducted by a pediatrician. Eight children were lost to follow-up.



STUDY III

In 39 children allergic to milk and/or egg and on an elimination diet, we performed 52 oral food challenges: 23 to milk and 29 to egg, (30 double-blind placebo-controlled, and 22 open standardized) when SPTs were ≤ 10 mm and SCORAD, ≤ 25.
We obtained blood samples in 44 of the challenges to analyze total and specific IgE.

Recipes
We developed recipes that were suitable for young children for use in both double-blind and open standardized oral food challenges to milk and egg. To make the recipes acceptable and easy to administer, egg was administered as an ingredient in a sponge cake and milk was added to the milk substitute usually given to the child. The recipes describe the exact amounts of supplied milk and egg in each dose.
Sensorial tests were conducted as a triangle test to ensure that active and placebo challenges were identical.[83, 95] The taste panel comprised 15 adults and 8 children; none had any known allergy to the included ingredients. The subjects were asked to detect a perceivable difference between three samples, one, two, three or none of which contained the active substance. All tests were served cool in medical pots that were coded for each test. The participants were allowed to rinse their mouth with water before and after testing. They were then asked to say which of the samples were the same and whether any differed from the others.

Food challenge
The children were allowed a light breakfast two hours prior to the challenge. Antihistamines had been withdrawn three days previously. Topical corticosteroids of low and medium class were allowed if required. The children were equipped with an

intravenous access during challenge.[83] Blood tests obtained to measure total and food-specific IgE were analyzed retrospectively after the challenge was performed. Successively increasing doses of the allergen were given in amounts of 0.1, 0.5, 5.0, 15, and 30 ml for milk and 0.1, 0.5, 1.5, 5, and 10 g for egg. The interval between each dose was 20 min.[83] Both a doctor and a nurse were present, and rescue medication was ready to be used in case generalized/severe allergic reactions occurred. The challenge was immediately stopped if objective clinical symptoms arose. The children were observed for two hours after the final dose was administered.[83] The food challenges were scored positive if objective clinical allergic reactions were observed. Clinical reactions within two hours after the final dose were defined as early, thereafter as late.[15,54] In the double-blind challenges, the interval between the two sessions was two weeks. The nurse contacted the family the following day and one week after the challenge to assess the occurrence of late reactions. In the DBPCFC, the code was broken after these two contacts. If neither early nor late allergic manifestations had appeared, the family was instructed to introduce the food in successively increasing amounts. The children were followed up after three months to assess the introduction of the foods and enquire whether symptoms of allergy had appeared.

Statistics
For statistical analysis, the Mann-Whitney U-test was used. Differences associated with *p* values of less than .05 (2-tailed) were considered significant.

Ethics
The Human Research Ethics Committees at the Faculty of Health Sciences, Linköping University, and at the Medical Faculty at Uppsala University approved the study. Informed consent was obtained from the children's parents.

STUDY IV

Ninety-four children (58 boys, 36 girls), with complete information and with urinary samples from two examinations, were selected from the total study group. The age of the 94 children at the first visit was 7.5 ± 5.2 months (mean ± SD) and at the second visit, 10 ± 5.4 months (mean ± SD).
The diagnosis of eczema was established using the criteria defined by Hanifin and Rajka, and the eczema was assessed with the SCORAD method. SPTs to cow's milk and egg were performed. A morning sample of urine was obtained. After 6-8 weeks, at a second visit, the SCORAD assessment was repeated, and another urinary sample was collected. The children's diet, according to Swedish recommendations for infants, did not contain food items with high levels of nitrite/nitrate, such as several vegetables (e.g. spinach, beetroot, rhubarb, fennel, celery) and smoked and salted meat products.

Method for measuring nitrite/nitrate in urine
In the urinary sample, the sum of nitrite and nitrate was measured as an indirect indicator of the NO production.[96] In short; the nitrite content was measured with a colorimetric method based on Griess reaction for nitrite. In a PBS-diluted sample,

nitrate was converted using nitrate reductase from Aspergillus.[97] Next, 50 µl of the diluted urine was mixed with 10 µl NADPH (1 µM) followed by 40 µl containing nitrate reductase (80 U/l, Roche, Basel, Switzerland), glucose-6-phosphate (500 µM) 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 µl sulfanilamide (1% in 5% phosphoric acid) and 100 µl naphtylethylenediamine (0.1%). The resultant color was read with a spectrophotometer (Vmax, Molecular Devices, Sunnyvale, CA) at 540 nm.

Statistics
For statistical evaluation, Student's t-test in the statistical software program SPSS 11 for Mac 0SX was used.

Ethics
The study was approved by the Human Research Ethics Committee at the Faculty of Health Science in Linköping and at the Medical Faculty at Uppsala University. Informed consent was obtained from the children's parents.

STUDY V

The study population consisted of 123 children evaluated from referral at < 2 years of age until follow-up at 4½ years of age, as described above in 'STUDIES III-V, The study population'.
At the 4½-year follow-up the children were assessed with respect to eczema using the SCORAD evaluation, and with SPT to detect sensitization to milk, egg and aeroallergens. We enquired about other allergic manifestations and clinical tolerance to foods.
The criteria used were:
- Asthma: any episode of wheezing if related to exposure to allergens or combined with AE, or at least three episodes of wheezing in the absence of atopy and exposure to allergens.
- AR: rhinitis at least twice after exposure to allergens and not related to infection.
- Gastrointestinal allergy: vomiting and/or diarrhea at least twice after intake of an offending food.

A pediatrician conducted the examination both at referral and at follow-up.

Statistics
For statistical analysis, the Chi2 test and the Mann-Whitney U-test were used. Differences associated with p values of less than .05 (2-tailed) were considered significant.

Ethics
The Human Research Ethics Committees at the Faculty of Health Sciences, Linköping University, and at the Medical Faculty at Uppsala University approved the study.
Informed consent was obtained from the children's parents.

RESULTS

STUDY I

A total of 1,152 children were subjected to skin prick testing over the study period. The skin tests were performed by the same two specially trained, experienced nurses and in a standardized manner.[62]
All tests involving food allergens were performed as prick-prick tests. Double-prick test, i.e. two tests of each allergen at the same session, was standard in our clinic at that time, although a single prick test was performed if the skin was highly affected by erythema or eczema. Milk and egg were always tested at the same session. Children with extensive eczema or other manifestations of allergy, (e.g. asthma) or with an ongoing respiratory infection, were examined and evaluated by a doctor before the test was performed.

Six cases of generalized reactions occurred, all in infants less than 6 months of age. They all had eczema, although the severity varied. Four of the children were breastfed only, one was given formula, and one was both breastfed and given formula. There was a family history of atopic disease in all cases. None of the children had previously had any wheezing episodes. Two children had an ongoing cold at the time of the skin prick test. The onset time of the generalized reaction was between a few to 20 minutes. In five of the cases, a positive test result for food allergy could be read. In one case the test result could not be read due to extensive skin reaction.
The six affected infants received immediate treatment by the nurses with antihistamine and/or epinephrine. They were examined without delay by a pediatrician, who added steroids to the treatment in three cases. The pediatrician made the clinical decision as to when the child could be dismissed from the ward after recovery.

Six cases of generalized reaction out of 1,152 tested children give an overall rate of 521 generalized reactions per 100,000 skin prick tests performed (0.52%). In the age group < 6 months, the corresponding figure was 6,522 per 100,000 (6.5%).

Age Group	Children Tested	Children with G.R.	Rates per 100,000	CI (95%)
< 6 months	92	6	6522	1476-11567
6-12 months	144	0		
1-19 years	926	0		
Total	**1152**	**6**	**521**	**105-937**

G.R. = Generalized reaction, CI = Confidence interval

STUDY II

At the pediatric clinic in Linköping, 224 children were tested with duplicate skin prick tests in 1997. Of those, 90 were young infants, i.e. <2 years of age.
In total, 1,087 duplicate tests were performed, resulting in 310 (28.5%) with duplicate positive test; 763 (70.2%) with duplicate negative test; and 14 (1.3%) with one positive and one negative test. Of 340 tests performed in infants younger than 2 years of age, 83 (24.4%) gave two positive results; 254 (74.7%) two negative; and 3 (0.9%) one positive and one negative. The test results with one positive and one negative included both food allergens (n=6) and inhalation allergens (n=8). It could be noted that of the 14 occasions with diverging results, the negative test result in 6 was below the cut-off limit for a positive result, but was still detectable, whereas 8 displayed a result that was totally negative (0 mm) and did not even express an erythema.

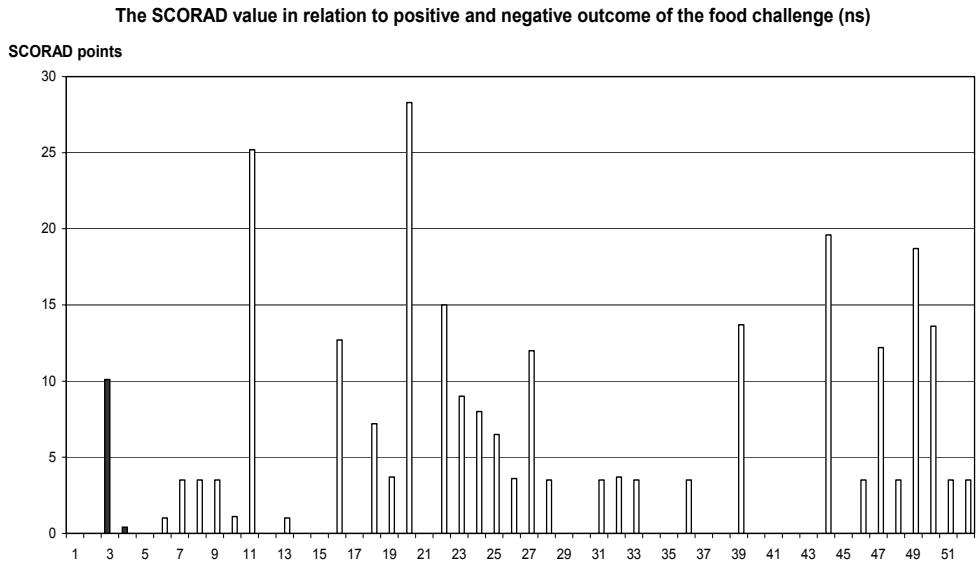
STUDY III

Clinical assessment
In the entire study group of 123 children, 78 were SPT-positive to foods and 45 were SPT-negative. Of the 78 milk- and/or egg-sensitized children 47 were assessed as food-allergic. These 47 children with food allergy constitute 38% of the 123 with eczema, which concurs well with previously presented figures.[14,15] In 39 of the 47 children we were able to proceed with the model for low-dose challenge.
These 39 children were 5 months old (median, range 1-23 months) at the first visit; 18 were girls and 21, boys. All 39 children fulfilled the criteria for atopic eczema according to Hanifin-Rajka and expressed SPT positivity to milk and/or egg. The children's age when challenged was 39 months (median, range 18-66 months).

Recipes and outcome of the *sensorial tests*
The recipes and the protocol for standardized open and DBPCFC were well accepted by the children. The sensorial tests performed by the taste panel did not show any differences in taste or appearance in the samples with or without the active substance.

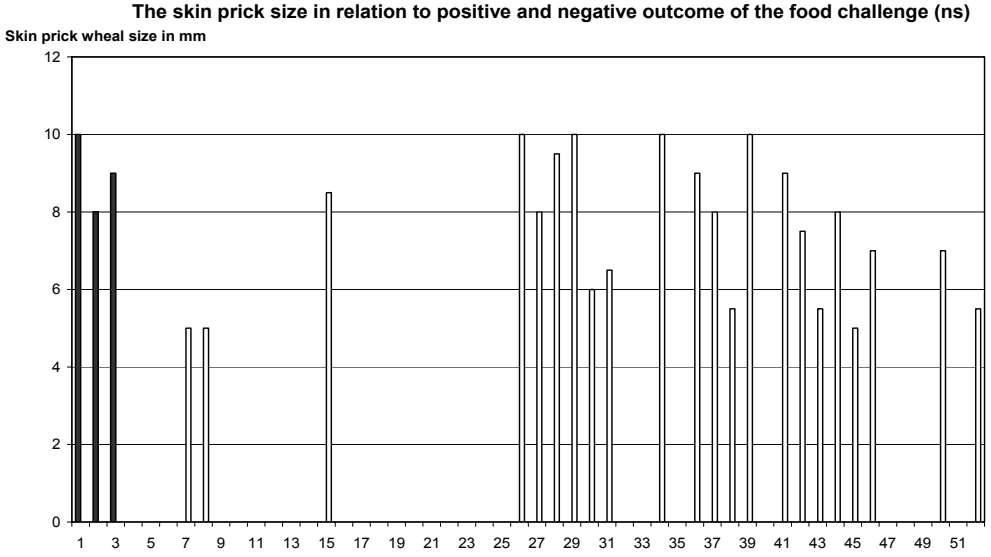
Outcome of food challenge
Four children had a positive challenge outcome (Tab.1). They were subjected to DBPCFC to milk and reacted on the first to third dose administered. There were no significant differences with respect to family history, associated atopic manifestations, nutritional supply, eczema severity, or skin prick test compared with the non-reacting children, but total and specific IgE values were significantly higher (Figure 1-4). One child had a negative SPT at the time of the challenge but expressed an SPT of 10.5 mm when re-tested two weeks after the challenge. This child had the highest value of the four for milk-specific IgE, 4.34 kU_A/L.

Fig 1.



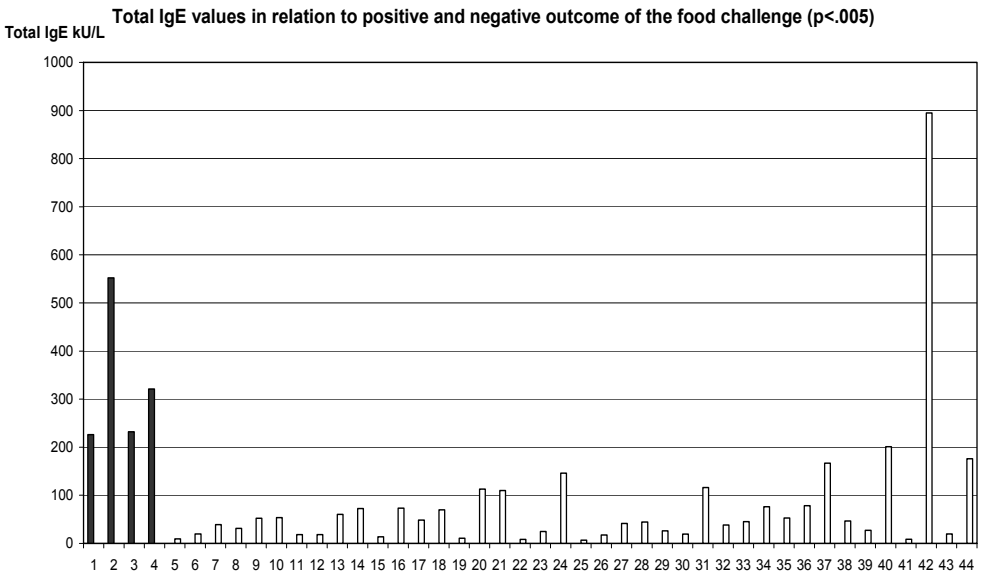
Bars no. 1-22 show SCORAD points when challenged with milk, and no. 23-52 show SCORAD points when challenged with egg. Bars no. 1-4 show children with positive outcome of the challenge, and no. 5-52 show the non-reacting children.

Fig 2.



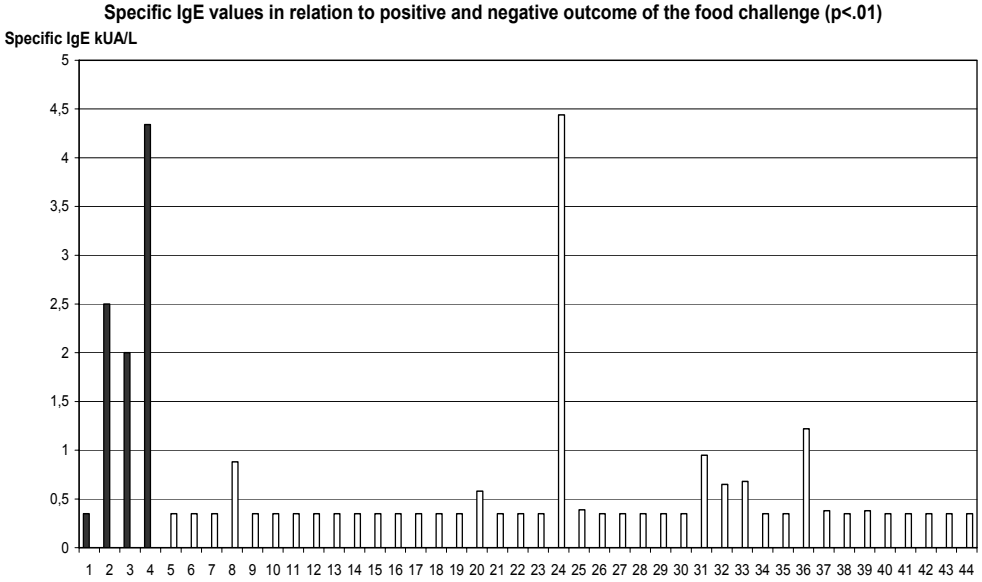
Bars no. 1-22 show SPT to milk wheal size when challenged with milk, and no. 23-52 show SPT to egg wheal size when challenged with egg. Bars no. 1-4 show children with positive outcome of the challenge, and no. 5-52 show the non-reacting children.

Fig 3.



Bars no. 1-20 show total IgE values when challenged with milk, and no. 21-44 show total IgE values when challenged with egg. Bars no. 1-4 show children with positive outcome to the challenge, and no. 5-44 show the non-reacting children.

Fig 4.



Bars no. 1-20 show specific IgE to milk when challenged with milk, and no. 21-44 show specific IgE to egg when challenged with egg. Bars no. 1-4 show children with positive outcome of the challenge, and no. 5-44 show the non-reacting children.

Tab.1

The four children with positive outcome of challenge

	Age months	SCORAD points	SPT mm	Total IgE kU/l	Spec IgE milk kU_A/l
Child 1	66	0	10	226	<0.35
Child 2	40	0	8	552	2.5
Child 3	39	10.1	9	232	2.0
Child 4	21	0.4	0	321	4.34

Post food challenge follow-up
At the three-month follow-up, three of the four children with a positive challenge outcome were still on a milk- and egg-free diet. Child nr 1 in the table had, at its parents' initiative, received small amounts of milk in its diet without problems. All but two of the non-reacting infants had successfully introduced the food into their diet without reactions.

STUDY IV

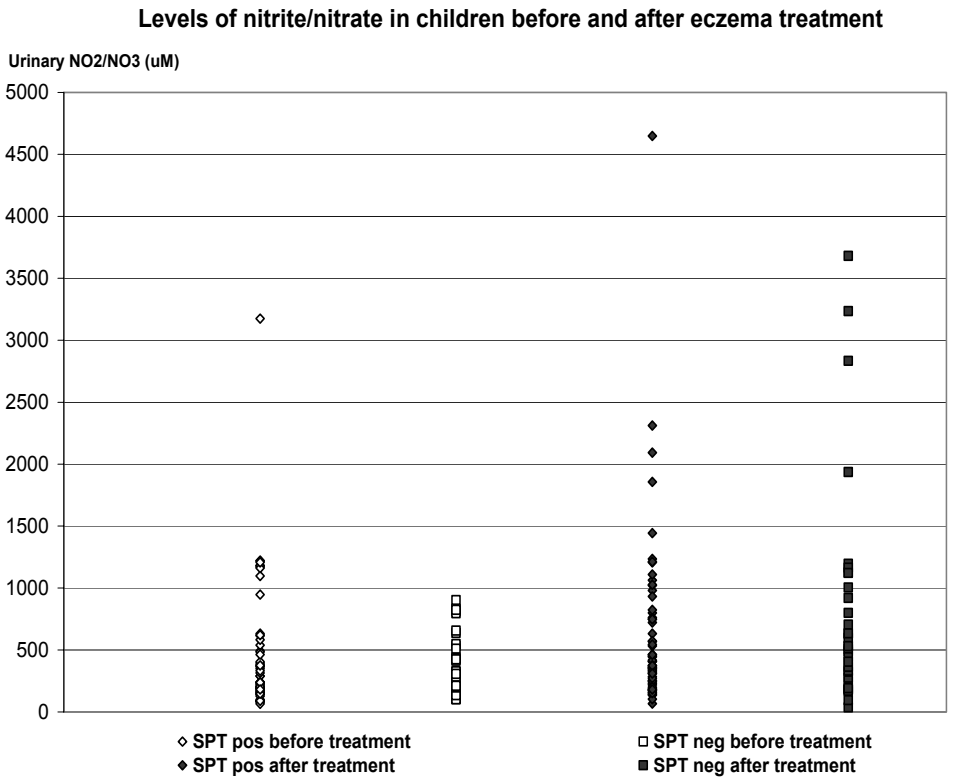
Skin prick test positivity
In 62/94 children, the SPTs to egg and/or milk were positive, whereas 32/94 children displayed negative SPTs.

Comparing nitrite/nitrate levels with age, atopic symptoms, eczema severity and nutrition
The majority of the children, 80/94 (85%), fulfilled the Hanifin-Rajka criteria for AE.
The SCORAD value for the whole group was 22.0 ± 6.0; 17.1; 0-77 (mean ± SD; median; range) at the first visit, and 11.6 ± 10.4; 9.0; 0-45.2 at the second visit (P<.001 for both paired and unpaired test).
For the SPT-positive children the values were 24.2 ± 17.8; 19.9; 0-77 at the first visit, and 11.7 ± 10.8; 9.1; 0-45.2 at the second (P<.001 for both paired and unpaired test). The corresponding figures for the SPT-negative group were 17.8 ± 14.5; 15.2; 0-50.8 at the first, and 11.2 ± 8.6; 9.0; 0-22.2 at the second visit (P<.05 unpaired, and P<.005 paired). Nitrite/nitrate levels and SCORAD value were not correlated.

When examined at the first visit, 52/94 children were still being breastfed, whereas 91/94 had been breastfed from birth until at least 2 months of age. Breastfeeding did not correlate with nitrite/nitrate value. Neither were nitrite/nitrate values influenced by age, presence of gastrointestinal symptoms, or airway symptoms.

Nitrite/nitrate in urine on the first and second occasions

The values for the whole group were 420 ± 428; 286; 65-3174 µM (mean ± SD; median; range) on the first, and 711 ± 775; 448; 36-4648 µM on the second occasion (P<.005 paired, and P<.002 unpaired test). In SPT-positive children, the levels were 436 ± 497; 238; 65-3174 µM, on the first, and 646 ± 705; 407; 68-4648 µM on the second occasion (ns). For SPT-negative children, the respective values were 387 ± 249; 316; 102-903 µM, and 840 ± 893; 530; 36-3680 µM (P<.01 for both paired and unpaired tests). There was no significant difference between the SPT-positive and SPT-negative group at the first or second visit. For the majority of children, 60/94, the values in the second measurement were higher than in the first.



STUDY V

At first visit

In 123 children referred because of eczema and suspected food allergy, 78 tested SPT positive to milk and/or egg, and 45 tested SPT negative. The Hanifin-Rajka criteria for atopic eczema were fulfilled in 75/78 (96%) in the SPT- positive children and in 25/45 (56%) in the SPT-negative children (p<.001).The groups differed significantly in severity of eczema scores, with more severe disease in the food-sensitized children, 19.9;0-77 (mean;range), compared with SPT-negative children 13.5;7.0-50.8 (p<.001). In SPT-positive children, 72/78 (93%) had a positive family history of atopy (first-degree relative), and 76/78 (97%) were breastfed. In the SPT-negative children, the results were 41/45 (91%) and 43/45 (96%), respectively (ns). There was no difference between the groups with respect to keeping pets. In 5/19 families with pets, the child suffered from airway symptoms. These 5 children were all SPT-positive. Tobacco smoking at home was reported by four families, all with SPT-positive children. Three of these children had airway symptoms as well as eczema. There was no significant difference between SPT-positive and SPT-negative children regarding presence of airway symptoms, 22/78 (28%) and 9/45 (20%), respectively.

At follow-up at 4½ years of age

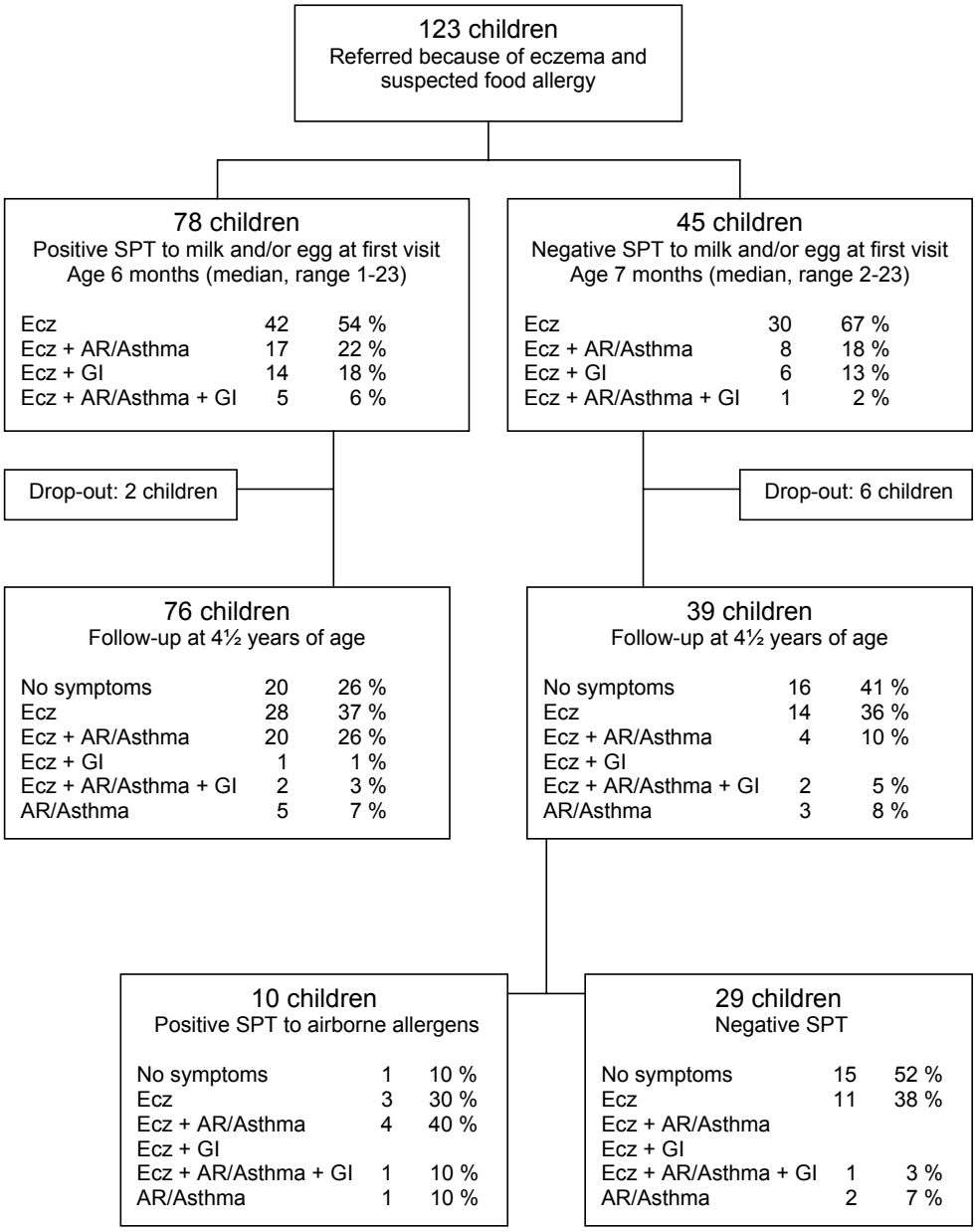
Significantly more children were still affected by eczema in the initially SPT-positive group compared with the children who remained SPT-negative at the 4½-year control, (p<.05).

In the initially SPT-positive group, 33/76 (43%) were still sensitized to milk and/or egg, expressing positive SPT. None of the SPT-negative group had become sensitized to food allergens (p< .001). All 39/39 children in the SPT-negative group were able to drink milk, and 38/39 (97%) could eat egg. One child avoided egg because it caused gastrointestinal symptoms. The corresponding figures for the SPT-positive group were 67/76 (88%) and 61/76 (80%), respectively, and for the food-allergic children 38/47 (81%) and 32/47 (68%). When comparing sensitized and food-allergic children with non-sensitized children, the differences were significant for both milk and egg, (p<.05 and p<.01).

SPT positivity to airborne allergens was expressed in 44/76 (58%) in the SPT-positive group; the corresponding figure in the SPT-negative group was 10/39 (26%), (p<.001). With respect to the presence of airway symptoms, there was no significant difference between the groups, 27/76 (36%) and 9/39 (23%). Nor was there any difference between the groups with respect to keeping pets. In 9/19 families with pets, the children suffered from asthma symptoms: seven from the SPT-positive group and two from the SPT-negative group. The four children living in homes in which tobacco was smoked all had asthma at the follow-up, and three were sensitized to airborne allergens.

With respect to the absence of atopic symptoms at follow-up, the difference between the initially SPT-positive group and the group who remained SPT-negative at the 4½-year follow-up was significant (p<.05).

Presence of eczema (Ecz), gastrointestinal symptoms (GI) and asthma/allergic rhinitis (AR) at referral and at the 4½-year follow-up.



DISCUSSION

STUDY I

Skin prick testing is a relatively easy way to test children for sensitization and is most often carried out without problems. We report six cases, < 6 months of age, expressing a generalized allergic reaction when skin prick tests were performed, whereas no such reaction was observed in children > 6 months of age. This indicates a higher risk of adverse reactions in very young infants.
The tests were applied in duplicate in four of the cases, which caused an extra allergen load on the limited surface of the small arm. Duplicate tests, like multiple skin tests, increase the risk of summation of the reactions and therefore also the risk of generalized reaction.
A number of precautions have previously been suggested related to the performance of skin prick tests: avoid skin prick tests when there are ongoing allergic reactions, perform skin prick tests only in the presence of both a nurse and a doctor and with rescue medicine at hand, and when using non-standardized extracts, the tests should only be performed under the supervision of a specialist with knowledge of the risks and precautions involved.[70]

We suggest increased precautions when testing infants of very young age. The testing should only take place in a setting where the safety precautions are optimal. To avoid a summation of reactions, we suggest the application of only one allergen at each visit, and no application in duplicate.
Despite the risk of adverse reactions, delaying the investigation is not recommended since early diagnosis will spare the child unnecessary suffering from the symptoms of allergy. The aim, however, should be to perform the test under optimal conditions.

STUDY II

Duplicate skin prick testing has previously been recommended because of reports of accidental negative tests, and the method has been used routinely in our department. However, because of six cases in 1996 to 1998, in which infants developed generalized allergic reactions in connection with the test situation, the routine has been altered, and since mid-1998, the infants are tested with single skin prick tests only.
When analyzing the test results from 1997, we found only 14 duplicate results with one positive and one negative wheal of the 1,087 tests performed (1.3%). In the youngest age group (<2 years), even fewer, 3 of 340 (0.9%), had an outcome of one positive/one negative test result.
In young infants, significant hyporeactivity has been reported and, therefore, smaller prick test wheals.[73] This suggests that a lower cut-off limit might have to be allowed in this age group for positive skin prick test results. If 2 mm were to be used as the limit for food allergens in the youngest age group, the outcome of the present study would be only 1 of 340 (0.3%), with one positive/one negative test result.

In children under 2 years of age, 89% of all tests performed are tests for food allergens. Because few standardized food-allergen extracts are available on the market, and those that are available are titrated in weight/volume, which is an unsatisfactory method of titration, fresh or frozen food specimens are recommended.[62,70,94] The allergen concentration is likely to vary in non-standardized extracts. However, applying non-standardized extracts does not seem to increase the test outcomes with diverging results between the wheals, which amounted to only 0.9% in the younger infants. The findings in this study support the use of single prick when performing the skin prick test.

STUDY III

We developed recipes and a protocol for low-dose oral food challenge, open standardized and DBPCFC. 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 when using unnatural forms of foods.

Our aim was to rule out children who might have an acute allergic reaction when exposed to small amounts of the food, and to identify the children for whom it would be possible to start introducing milk and egg in small, but successively increasing, amounts.

Opinions differ concerning when SPT conclusively predicts a positive challenge. The traditional cut-off level of $\geq$ 3 mm or greater has a great potential for diagnostic error[56], and different cut-off values have been proposed in several studies, varying from 5 to 12.5 mm for milk and from 4 to 13 mm for egg.[72,86,88] Cutaneous reactivity might, however, vary with age, time of day, season, and the patient's gender. Therefore, different cut-off values are likely to be required for different subpopulations of children.[41,56,88] Thus, when contemplating a challenge, SPT size and IgE values may be useful tools, but it is more important to give reasonable consideration to the severity of previous reactions and the kind of allergen involved.[41]

In the present study we set the cut-off level of SPT to wheal sizes $\leq$10 mm for milk and egg. Four children who were SPT-positive to milk had a wheal size of >8 mm; 3 of them had a positive challenge outcome. In children challenged with egg, 13 had an SPT wheal size of > 7 mm, and none of them reacted at challenge. The children were challenged with baked, not raw, egg, which may explain the greater number of children who were able to eat egg.

The 4 children who had a positive outcome of the challenge reacted to very small amounts of the allergen. All 4 underwent a double-blind challenge to milk. There were no significant differences with respect to family history, associated atopic manifestations, nutritional supply, SCORAD points, or SPT positivity compared with the non-reacting children, but in total and specific IgE the values were significantly higher. In some of the challenges, the children might already have achieved full tolerance, and in others tolerance development was ongoing. Our model does not distinguish between these conditions.

For the non-reacting children, the food in question was introduced without problems in all but two cases: one child due to the difficulties of having two separate diets for twin

brothers, and the other because of suspected non-IgE-mediated hypersensitivity. In some of the cases, the parents reported episodes with somewhat aggravated eczema at follow-up. These resolved themselves spontaneously, however, and the introduction of the food was carried on without interruption.

Because tolerance is likely to develop gradually, parents may notice long before the child tolerates normal amounts of milk and egg that an intake of small amounts of milk and egg in cooked food no longer produces any symptoms. The model for low-dose challenge used in this study can facilitate the re-/introduction of milk and egg in young sensitized children outgrowing their allergy. It is, however, important to inform the parents of the amount of the food the child has been tested with and that a negative outcome when challenged with cooked egg does not exclude a possible reaction to raw egg. We instructed the parents to start with the amount used in the challenge as the daily intake and only successively to increase the amounts. Support from the allergy nurse via regular telephone contact encouraged the parents to continue introducing the foods at home and successively reach an age-estimated daily intake. The introduction of the foods at home following a negative challenge under the telephone supervision of an experienced allergy nurse may replace the regular open challenge after a negative double-blind challenge.

STUDY IV

As expected, the treatment improved the eczema, as assessed by decreased eczema scoring. However, contrary to our expectations, urinary NO breakdown products increased significantly in parallel with the eczema improvement, and there was no correlation between eczema severity and urinary nitrite/nitrate levels. This is in contrast to a previous study of children with eczema. They 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.[98] However, that study was performed with serum samples, and the children were older (mean age 2.2 years). This presumably means that their eczema is of a more chronic nature. No information was provided regarding diet, i.e. intake of food rich in nitrite/nitrate, or sensitizations to foods requiring elimination diets.

In this study, urinary excretion of NO products increased significantly after treatment, from 420 ± 428 μM (mean ± SD) on the first occasion to 711 ± 775 μM at the second investigation. For reference, a previous study measured levels in healthy children of the same age at 1174 ± 116 μM (mean ± SEM).[99] A possible explanation for increasing levels of nitrite/nitrate would be large consumption of food products containing nitrite/nitrate. This is not a likely explanation, as parents in Sweden are advised not to serve these food items to their infants. Another source of NO production in the body might be untreated asthma, as several studies show the usefulness of analyzing exhaled NO products as a sign of airway inflammation.[100,101] Among the 94 children in this study, 20 were diagnosed with asthma, and their urinary nitrite/nitrate levels did not differ from those of children without airway problems.

An inflammatory reaction, as in eczema, is thought to result in an activation of the stress system, which induces a Th1/Th2 shift, to provide protection from systemic "overshooting" with Th1-induced proinflammatory cytokines and elevated levels of toxic NO products.[36] However, the inhibition of NO may cause increased intestinal inflammation with mast cell degranulation and increased permeability, and could reduce the possible positive effects of NO, which may be important for skin and intestinal mucosa healing.[35,36]
Further, the immune modulating effects of NO have recently been studied in asthma by in vitro studies of human bronchial epithelial cells.[37] It is generally assumed that NO only has a harmful influence in asthma, by selective downregulation of Th1 responses. However, the cited study showed that NO can limit in vitro expansion of both CD4+ Th1 and Th2 cells and reverse the Th2 to Th1 shift that follows on treatment of inflammation.[37] Human epithelial cells upregulate NOS, which causes NO release, which in turn inhibits Th1 and Th2 proliferation. When NOS is specifically blocked, the T cell proliferation is shown to be completely restored. By this feedback loop, the organism may putatively protect the airways from overwhelming inflammatory response after allergen exposure.[37] Interestingly, the authors suggest an individual variation in the efficiency of this feedback loop, explaining the fact that only some of the children sensitized to aeroallergens develop asthma.[37]

Compared with children with celiac disease, we found that the nitrite/nitrate levels at the second visit, 711 ± 775 μM (mean ± SD) in this study, is lower than the values observed in celiac children on a gluten-free diet, i.e. when healing of the intestinal mucosa has been demonstrated in small-intestinal biopsy. For these children, the nitrite/nitrate values was 1078 ± 1084 μM (mean ± SD).[102] Children with active CD have been shown to have much higher nitrite/nitrate values, 4147 ± 1102 μM (mean ± SEM), when the mucosa is inflamed.[99] However, as CD is not an atopic condition, the proposed mechanism with inflammation-induced Th1/Th2 shift is not applicable, and high levels of nitrite/nitrate are likely to result from the inflammatory state in the intestine in active CD.

We hypothesize that our findings with low levels of NO in children with active eczema and increased levels after treatment might be explained by a similar mechanism with upregulation of iNOS, as observed in human epithelial cells in asthma. The majority of the children in our study displayed elevated NO levels after treatment, but not all, which might be explained by individual variations in the feedback system as previously suggested.[37] Further investigations of these children at a higher age will reveal if these individual variations persist.

STUDY V

We followed 123 children from referral because of eczema and suspected food allergy until 4½ years of age. Of the 78 milk and/or egg sensitized children 47 were assessed as food-allergic. These 47 children with food allergy constitute 38% of the 123 with eczema, which concurs well with previously presented figures.[14-16]
It has been described that food allergy contributes to the severity of eczema.[9] In the present study a difference in severity between food sensitized and non-food-sensitized children was confirmed in infants (<2 years), but not later in childhood (4½ years).
Complete remission of AE by 3 years of age has previously been shown in 43% of children with early AE.[103] This was demonstrated in a population-based study evaluating a birth cohort of 1,314 children. In the present study, in children referred to pediatric clinics, the remission was somewhat lower, 38% for the whole group and 33% in SPT-positive children. However, in SPT-negative children the complete remission was 49%. The findings concur with previous reports that the prognosis of AE is determined by the presence of sensitization.[103]

Most children will outgrow their allergy to milk. Different results have been shown with respect to tolerance achievement in IgE-mediated milk allergy, including 76% by age 3[44], 56% by age 4[45], 78% by age 6[45], 38% by age 7[46], and 57 % by age 8.[47] In the present study, 81% of early sensitized and previously milk-allergic children achieved tolerance and were drinking milk at 4½ years of age. This is more than the other studies have shown and might have been achieved because we performed challenges early in children expressing SPT wheal sizes up to and including 10 mm. Two studies on children outgrowing food allergy to egg have demonstrated tolerance achievement in 30-44% by school age.[48,49] In the present study, 68% were tolerant at 4½ years of age. These children, expressing SPT wheal size up to and including 10 mm, were challenged early, but with baked, not raw, egg, which may explain the greater number of tolerant children.

The concept of the atopic march suggests that approximately 80 % of children with AE will eventually develop asthma and/or AR, with many outgrowing their AE with the onset of respiratory allergy.[9,19,20] Sensitization to aeroallergens has previously been demonstrated in 48% of milk-allergic children at age 3.[44] We found a significant difference between the children early sensitized to foods compared with SPT-negative children in sensitization to airborne allergens at the 4½ year follow-up, 58% and 26% respectively (p<.001), but found no difference in symptoms of asthma/AR. The higher rate of sensitization suggests, however, that on following up this group at school age, the number with allergic airway symptoms will increase and could reach the 80% previously reported. However, the low rate of tobacco smoking in the families in this study might favor the prognosis and result in a lower rate of children developing asthma/AR.[22]

CONCLUSIONS

STUDY I

Possible risk factors for generalized allergic reactions in connection with skin prick testing might be young age, i.e. < 6 months of age, summation of reactions when applying several allergens or allergens in duplicate at the same session, active and spread eczema, and the use of non-standardized extracts. The risk of generalized reactions after skin prick test with fresh food specimens in young infants ought therefore to be acknowledged and should lead to increased precautions when performing the skin prick test.

STUDY II

Applying the allergens in duplicate when performing the skin prick test has previously been recommended to minimize false negative outcomes. We found that only 1.3% of the results in duplicate skin prick tests had diverging outcomes, and in infants < 2 months, even less, 0.9%. Considering the risk of inducing a summation of the reactions, and thereby a generalized allergic reaction, when applying an extra allergen load on the limited surface of the small arm, we conclude that the results of this study justify using single prick test, at least in the youngest age group and likely when testing children of all ages.

STUDY III

Low-dose standardized open and double-blind placebo-controlled food challenge in young children can facilitate the introduction of small amounts of egg and milk into the diet during tolerance development. It is a relief for families when their child can tolerate small amounts of milk and egg in bread or cooked food, even if larger doses may still cause symptoms, sparing the parents the fear that the child might have a severe allergic reaction if accidentally exposed to small amounts of the food.

STUDY IV

Urinary NO breakdown products increased significantly after treatment of eczema in parallel with improvement of the skin. This increase might be due to a Th2/Th1 shift induced by the eczema treatment; i.e. by the amelioration of the inflammation present in the eczematous skin. Individual variation in NO-induced-feedback downregulation of Th1 and Th2 proliferation might explain the variations in NO response.

STUDY V

The prognosis for achieving clinical tolerance was very good in children allergic to milk and egg. We presume that our active challenge procedure, despite remaining moderate positive SPT, contributed to the favorable prognosis. In all children the eczema improved, with the highest remission rate in non-sensitized children. Children sensitized to foods became significantly more often sensitized to aeroallergens. However, the rate of asthma/AR in children sensitized to aeroallergens was low.

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Paper I

Skin prick tests may give generalized allergic reactions in infants

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Background: Skin prick testing, a widely used method of studying sensitization, is usually considered quick, pedagogic, and relatively inexpensive. Previous studies have shown very few negative reactions and no fatalities. In contrast, both anaphylaxis and death have been reported as a result of intracutaneous tests.

Objective: To examine detailed case studies of generalized allergic reactions in connection with skin prick testing in order to identify possible risk factors and thereby increase the safety of the test procedure.

Method: A retrospective study of medical records of six cases with generalized allergic reaction occurring during the study period 1996–1998 at the Pediatric Clinic, University Hospital of Linköping, Sweden. Data about the total number of children tested during the period were collected from the clinic's database.

Results: All six cases with generalized reactions were infants <6 months who showed positive skin prick tests to fresh food specimen. Other common features were active eczema and a family history of allergic disease. All infants received prompt treatment and recovered well. The overall rate of generalized reactions was 521 per 100,000 tested children. In the age group <6 months, the corresponding figure was 6522 per 100,000.

Conclusion: The risk of generalized reactions after skin prick test with fresh food specimens in young children ought to be acknowledged and should lead to increased precautions when performing the test.

Ann Allergy Asthma Immunol 2000;85:457–460.

INTRODUCTION

Skin prick testing is a widely used diagnostic tool when studying IgE-mediated hypersensitivity. The method, used extensively in children, has several advantages. Reactions can be read in 15 minutes, making it quick. Parents can see the reaction of the positive test result on the skin. Finally, it is relatively inexpensive compared with serologic methods for allergy investigation.

The method, however, also has disadvantages. It takes an experienced tester to give reliable results. Even with a very experienced user, the result will vary considerably because of differences in technique and quality of extracts, with standardized extracts giving the highest efficiency.[1,2] Further, the interpretation of the positive test requires skill and experience because a positive test is not synonymous with allergic disease.

Two different methods of allergy skin tests have been widely used. One is the intracutaneous method, which applies the allergen more deeply. The other is the skin prick test, which gives a more superficial application of the allergen in the skin.

The skin test methods have been extensively studied with regard to adverse reactions. Lin et al reported two cases of generalized allergic reaction when performing intracutaneous tests, whereas in the same study 10,400 skin prick tests showed no negative reactions.[3] In another study of lethal reactions to skin testing or hyposensitization, Lockey et al showed two cases of deaths in connection with skin testing. In all cases, intracutaneous techniques were used. No lethal reaction has been reported after skin prick tests.[4]

Skin prick testing is therefore considered to be a safe procedure. Turkeltaub et al reported minor adverse reactions occurring in a maximum of 0.49% of the tested patients.[5] Nevertheless, the skin prick test can induce systemic reactions in highly sensitive patients, even if such reactions are rare. Two cases of anaphylactic reactions in adults were reported in 1995 by Novembre et al after skin prick test using fresh food as the test substance.[6] Furthermore, Valyasevi et al, in their recent study of over 18,000 patients, reported six systemic reactions when skin testing was performed. No systemic reaction was severe. Five of the cases reacted after skin prick tests. The remaining reaction followed an intradermal skin test. None of the reacting patients were tested for food allergens.[7]

The interest in safety precautions for the skin prick test has been further increased by the fact that investigators have lost their former respect for skin testing as skin prick tests have largely replaced intradermal testing. For instance, nowadays the test is sometimes also used in settings other than hospitals, eg, primary health care centers.

In the present study, covering 1,152 skin prick tests performed during 1996–1998 in children 0 to 19 years of age, we report six cases of generalized reactions, all of which occurred in infants less than 6 months of age. We studied in detail the history of the cases observed to see whether the children shared any common features that could be regarded as risk factors. Identifying

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such factors could help to make the procedure safer.

METHODS AND MATERIALS

The present study is a retrospective analysis of medical records of six infants, all of whom developed generalized allergic reaction in connection with skin prick tests 1996–1998. Tests were performed on the babies at the Pediatric Clinic at the University Hospital in Linköping, Sweden. Information about the total number of tests were performed during the period and the distribution according to age was collected from a computerized database at the clinic.

Procedure for Skin Prick Testing

Skin prick tests were performed by two trained and experienced nurses. The volar aspect of the arm was used as the test area. The skin was marked with a ballpoint pen for the allergens to be tested. All tests involving food allergens were performed as prick-prick tests, ie, the tip of the lancet was first dipped in the fresh or frozen food specimens. For milk, fresh low-fat milk was used and for egg, frozen egg white. The tip of the lancet was then pressed at right angles against the skin surface for one second using the volar aspect of the finger tip. A metallic lancet with a 1-mm tip (ALK) was used to prick the skin. As a negative control, the skin was pricked with a clean lancet. The positive control was histamine HCL 10 mg/mL. The reaction was read 15 minutes after the test was finished.

Double-prick test, ie, two tests of each allergen at the same session, was standard, although a single prick test was performed if the skin was highly affected by erythema or eczema. Milk and egg were always tested at the same session. This is because it is common to find positive results to egg several months before milk, even though the child reacts clinically to both foods. The skin prick test was regarded as positive when the mean diameter (= half of the sum of the largest diameter and its perpendicular) of the wheal was at least 3 mm.

Children with extensive eczema or other manifestations of allergy, (eg, asthma) or with an ongoing respiratory infection were examined and evaluated by a doctor before the test was performed.

In the event of an immediate systemic reaction to the skin test, the nurse who performed the skin test was responsible for administering appropriate treatment until the doctor arrived.

The six infants received immediate treatment with antihistamine and/or epinephrine. They were examined without delay by a pediatrician, who added steroids to the treatment in three cases. The pediatrician made the clinical decision as to when the child could be dismissed from the ward after recovery.

RESULTS

Six cases of generalized reactions to skin prick tests were reported between 1996 and 1998. Information about the children is summarized in Table 1. The outcomes of the skin prick tests appear in Table 2. Total number of tested children, distribution according to age, and the rate of cases with generalized allergic reactions are summarized in Table 3.

All six generalized reactions affected babies less than 6 months of age. All had eczema, although the severity varied. Four of the children were breastfed only, one was given formula, and one was both breastfed and given formula. There was a family history of atopic disease in all cases. None of the children had previously had any wheezing episodes. Two children had an ongoing cold at the time of the skin prick test. The onset time of the generalized reaction was between a few to 20 minutes.

In five of the cases, a positive test result for food allergy could be read. In one case the test result was impossible to read due to extensive skin reaction. Six cases of generalized reaction out of 1,152 tested children give an overall rate of 521 generalized reactions per 100,000 skin prick tests performed. In the age group <6 months, the corresponding figure was 6522 per 100,000.

Table 1. Summarized Information About the Six Children who Developed a Generalized Reaction at Skin Prick Tests

Child	Sex	Age at Test	Heredity	Food	Eczema†	Infection‡	Year
1	F	5 months	2 sibs	Breastmilk	Mild to moderate	No	1996
2	M	2.5 months	m. f.	Breastmilk	Extensive, severe	Yes	1997
3	M	3 months	m.	Formula	Extensive	No	1998
4	M	3.5 months	m. f. s.	Breastmilk	Mild to moderate	No	1998
5	M	5 months	s.	Breastmilk	Mild to moderate	Yes	1998
6	F	5 months	f.	Breastmilk + Formula	Extensive, severe	No	1998

Abbreviations: F = female, M = male, m = mother, f = father, s = sister, and sibs = siblings.
† Eczema means active eczema when the test was performed.
‡ Infection means signs of upper respiratory tract infection.

Table 2. Summarized Information About Allergens Applied at Skin Prick Tests, Positive Test Results, the Acute Symptoms Noted and Treatments Given Against the Systemic Reaction

Child	SPT Applied	Histamine Wheal	Positive results of SPT	Symptoms	Treatments
1	Egg, milk, fish, c. hyd.	6.0	Egg 1 10.0, Egg 2 9.5, Milk 1 7.0, Milk 2 7.0, Fish 1 4.5, Fish 2 4.5.	Urticaria, rhonchi	0.3 mg epinephrine SC 2.5 mg cetirizinidih. PO 5 mg betamethason PO
2	Egg, milk	Not readable due to the reaction.	Not readable due to the reaction.	Generalized erythema	0.15 mg epinephrine SC 0.125 mg clemastine PO
3	Egg, milk	20.0	Egg 1 4.5, Egg 2 5.5, Milk 1 4.0, Milk 2 3.0.	Generalized erythema, crepitations	0.15 mg clemastine PO
4	Egg, milk, fish, c.hyd.	7.0	Milk 9.5.	Generalized erythema	0.175 mg clemastine PO
5	Egg, milk.	5.0	Egg 1 8.0, Egg 2 7.5, Milk 1 7.5, Milk 2 5.0.	Urticaria, hoarseness, cough	0.1 mg epinephrine SC 0.2 mg clemastine PO 5 mg betamethasone PO
6	Egg, milk, c.hyd., w.hyd.	7.0	Egg 11.5, Milk 8.0.	Generalized erythema	0.35 mg clemastine PO 4 mg betamethasone PO

Abbreviations: SPT = skin prick tests, C.hyd. = casein hydrolysate, and W.hyd. = whey hydrolysate.
Positive results = mean wheal size in millimeter.
Urticaria means urticae over the whole body, not only locally.
Generalized erythema means an erythema over the whole body surface with severe itching but no definite urticae.

Table 3. Summarized Information About Total Number of Children Tested 1996–1998, Distribution According to Age and Generalized Reaction Rates

Age Group	Children Tested	Children with G.R.	Rates per 100,000	CI (95%)
<6 months	92	6	6522	1476–11567
6–12 months	144	0		
1–19 years	926	0		
Total	**1152**	**6**	**521**	**105–937**

G.R. = generalized reaction and CI = confidence intervals.

DISCUSSION

Skin prick testing is a relatively easy way to test children for allergy. It is most often carried out without problems. In the present study, however, we report six cases demonstrating a generalized reaction. During the study period, 6.5% had a generalized reaction in the age group <6 months. The figure for all tested children during the period was 0.52%. This indicates a higher risk of adverse reactions in very young infants than previous studies have shown. There are, however, limitations when comparing different studies. In some studies standardized extracts have been used. In others, including ours, non-commercial products have been chosen.[6] We used fresh foods as test substances. This is because very few standardized food-allergen extracts are available and many commercial food-allergen extracts are titrated in weight/volume, which is a very unsatisfactory method of titration.[6,8] Despite the limitations adherent to a retrospective study, the following common features were observed among all the six cases: active eczema, age below 6 months, positive reactions to food items, and a family history of allergic disease. The first three are naturally interrelated. Eczema is a dominant reason for investigating allergic disease in this age group, and foods are the main suspects of sensitization. The test was applied in duplicate to four of the cases, which caused an extra allergen load on the limited surface of the small arm. Duplicate tests, like multiple skin tests, increase the risk of summation of the reactions and therefore also the risk of generalized reaction. All six cases were infants < 6 months of age. No adverse reaction in connection with skin prick test has been observed at our clinic in children >6 months of age. Young age should therefore be considered a risk factor for developing generalized allergic reactions when undergoing skin prick tests.

Dreborg has previously suggested a number of precautions. If possible, avoid skin prick tests when there are ongoing allergic reactions. Perform skin prick tests only in the presence of

both a nurse and a doctor who have epinephrine at hand. Finally, skin prick tests using nonstandardized extracts should only be performed under the supervision of a specialist with knowledge of the risks and precautions involved in such testing.[9]

We suggest increased precautions when testing infants of very young age, especially if the child is affected by an extensive eczema. If testing is necessary, it should only take place in a setting where the safety precautions are optimal. To avoid a summation of reactions, we suggest the application of only one allergen at each visit, and no application in duplicate.

Despite the risk of adverse reactions, delaying the investigation is not recommended since early diagnosis will spare the child unnecessary suffering from the symptoms of allergy. The aim, however, should be to perform the test under optimal conditions.

A larger, prospective study on the safety of skin prick testing is currently in progress in southeastern Sweden. We hope that this will show that skin prick tests are a safe and valuable method if used with care.

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Paper II

Skin prick test in duplicate: is it necessary?

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Background: Duplicate skin prick testing has previously been recommended because of reports that accidental negative tests are common. However, duplicate tests also mean an extra allergen load, which may increase the risk of inducing a generalized reaction at the test situation, at least in the youngest infants.

Objective: To investigate whether the occurrence of both a positive and negative test result is a common feature when performing duplicate skin prick tests and can therefore justify the duplicate method.

Methods: A retrospective analysis of all skin prick tests performed in duplicate at the pediatric clinic at University Hospital in Linköping, Sweden, in 1997.

Results: Of 1,087 skin prick tests, 14 resulted in one positive and one negative test, or 1.3%. The corresponding figure in the youngest age group, (ie, <2 years of age) was 3 of 340 (0.9%).

Conclusions: Considering the risk of inducing a summation of the reactions, and thereby a generalized allergic reaction, when applying an extra allergen load on the limited surface of the small arm, we conclude that the results of this study justify using single prick test, at least in the youngest age group and probably when testing children of all ages.

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INTRODUCTION

Allergy is an increasing problem in the pediatric population, and recent studies report allergic manifestations in >40% of Swedish schoolchildren.[1] In early childhood, food allergies dominate, occurring in 3% to 5% of all children.[2]

When studying IgE-mediated hypersensibility, skin prick testing is a widely used diagnostic tool. The method is usually considered quick, pedagogic, and relatively inexpensive. However, it takes an experienced tester to produce reliable results. Differences in technique and quality of extracts can influence the results. Further, the interpretation of the test result requires skill and experience, as a positive test is not synonymous with allergic disease. However, the larger the test reaction, the more likely the clinical significance.[3]

Skin prick testing is considered to be a safe procedure. No anaphylactic reactions after skin prick test were observed by Turkeltaub et al,[4] and adverse reactions were very uncommon. Nevertheless, the skin prick test can induce systemic reactions in highly sensitive patients.[3,5,6] We have recently reported six cases of generalized reactions, all in children younger than 6 months of age, after skin prick tests when duplicate tests were standard in our clinic.[7]

When interpreting the results of skin prick test, a mean wheal diameter of 3 mm is commonly used as a practical lower limit for positivity.[6,8,9]

Wheals of 1 to 2 mm in diameter might be induced by pricking the skin, even with a dry needle.[10] However, a clear and significant hyporeactivity to histamine has been observed in infants, especially before the age of 6 months. The same study confirms, however, that skin prick test can be performed and interpreted without difficulties in infants after the age of 3 months, bearing in mind that the prick test wheals might be smaller.[11]

Because very young children are not likely to be sensitized to as many allergens as older children, fewer prick tests need to be performed. Sensitization is most apt to reflect exposure to allergens encountered in earliest life, primarily foods, but also dust mites, indoor molds, and animal danders.[3]

Performing duplicate tests with each solution at the test situation has previously been recommended as it has been reported that accidental negative tests are common. Dreborg et al[12] have demonstrated that even if the cutoff limit of 3 mm is used, many patients react with both positive and negative tests to the same concentration of the test substance. As many as 18 of 20 patients (90%) showed both positive and negative test results when tested for timothy and dog with an allergen concentration of 160 BU/mL. With an allergen concentration of 800 BU/mL, 5 patients of 20 gave both positive and negative responses. Thus duplicate tests have been recommended when screening for sensitization to avoid negative results.[12] Recent reports, however, recommend single skin tests in very young infants to minimize the allergen load at the test situation.[7,9]

One reason for using duplicate tests is the possibility of difference in skin sensibility between different parts of the forearm. However, several studies have shown that there were no differences in the wheal when the tests were performed on one arm and both arms, or proximally and distally in the test area on one arm.[13–15]

In this study, covering all duplicate tests performed in 1997 at the pediatric clinic in Linköping, we investigate whether the occurrence of both positive and negative test results is a common feature and can justify the use of duplicate tests in infants where an extra allergen load on the limited surface of the small arm may increase the risk of summation of the reactions and, therefore, the risk of generalized reaction.

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MATERIALS AND METHODS

The present study is a retrospective analysis of all skin prick tests performed in duplicate at the pediatric clinic at University Hospital in Linköping, Sweden in 1997. Tests performed with the single prick method (n = 33) were excluded from the study.

All test results are kept in the medical record of each child. In the study, all wheals have been measured, and mean diameter has been registered. We also registered whether the outcome was two positive, two negative, or one positive and one negative test result.

The skin prick test was considered positive when the mean diameter (= half the sum of the largest diameter and its perpendicular) was at least 3 mm for foods, and at least 2 mm for inhalation allergens. If the wheal of the histamine reference solution was <3 mm, a wheal the same size as, or larger than, the positive control would also be accepted as positive.[8]

The skin tests were performed by the same two specially trained and very experienced nurses in a standardized manner. The volar aspect of the arm was used as test area, and the skin was marked with a ballpoint pen for the allergens to be tested.[9] All tests involving food allergens were performed as prick-puncture tests.[3] The tip of the lancet was first dipped in the test solution or the fresh or frozen food specimens. When using inhalant allergens, a drop of test solution (ALK-Abéllo, Hørsholm, Denmark) was placed close to the respective mark on the skin. The tip of the lancet was then pressed at a right angle against the skin surface for 1 second using the volar aspect of the finger tip. The lancet used for pricking the skin was a metallic lancet with a 1-mm tip. As a negative control, a prick with a clean lancet was used. The positive control was histamine HCl 10 mg/mL. The reaction was read 15 minutes after the test was finished. The test results were documented by first outlining the wheal with a pen and then transferring the result to cellotape. The results was stored in the child's record. The mean diameter (the longest and the midpoint orthogonal) was measured with a transparent plastic ruler by the same investigator for all measurements.[9]

Statistics

Confidence interval 95% for one proportion was calculated according to Altman.[16]

RESULTS

At the pediatric clinic in Linköping, a total of 224 children were tested with duplicate skin prick tests in 1997. Of these, 138 (62%) were male and 86 (38%) were female. There were 90 very young infants, ie, <2 years of age, 59 (66%) male and 31 (34%) female.

In total, 1,087 duplicate tests were performed, resulting in 310 (28.5%) with duplicate positive test; 763 (70.2%) with duplicate negative test; and 14 (1.3%, 95% confidence interval 0.6 to 1.9%) with one positive and one negative test. (Fig 1)

Of 340 tests performed on infants younger than 2 years of age, 83 (24.4%) gave two positive results; 254 (74.7%), two negative; and 3 (0.9%, 95% confidence interval 0.2 to 2.6%), one positive and one negative. (Fig. 2)

The skin prick tests comprised both food and inhalation allergens. Of the 1,087 tests, 566 (52%) were food and 521 (48%) were inhalation allergens. For the infants <2 years only, the corresponding figures were 303 of 340 (89%) and 37 of 340 (11%), respectively.

The test results with one positive and one negative included both food allergens (N = 6) and inhalation allergens (N = 8). It could be noted that of the 14 results, the negative test result in 6 was below the cutoff limit for a positive result (ie, 3 mm for food allergens and 2 mm for inhalation allergens, but was still detectable), whereas 8 displayed a result that was totally negative (0 mm; Table 1).

A total of 13 infants displayed one positive and one negative test result, one of them having two tests with discordant results. All but one had a family history of allergic disease. Eight presented with eczema, 7 with asthma, 3 with eye/nose problems, 2 with symptoms from the mouth/throat, 2 with urticaria, and 1 with erythema. In the youngest age group (ie, <2 years

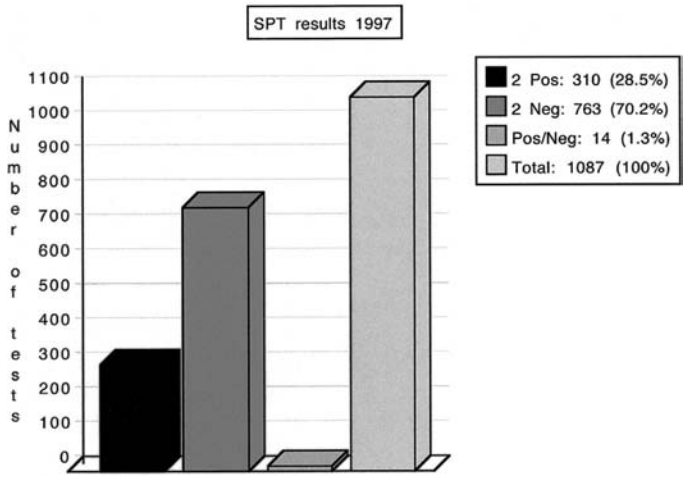

Figure 1. In total, 1,087 duplicate tests were performed in children 0 to 18 years of age in 1997. The figure shows the relationship among the results of duplicate positive, duplicate negative, and diverging results with one positive and one negative wheal. Fourteen of 1,087 tests show diverging results, corresponding to 1.3% (95% confidence interval 0.6 to 1.09%).

of age) all 3 had eczema, and 1 child also had asthma. No other symptoms were expressed in this group.

Four of the older children had presented with more generalized allergic reactions, including: 1) urticaria and itch in the throat when eating peanut. This child was tested for milk, egg and peanut. The result was positive for milk and egg as well as one positive and one negative for peanut. 2) Generalized erythema and itch in the throat when eating peanut. The test was performed for peanut, hazelnut, pollen, and cat, with a positive result for cat, one positive and one negative for peanut, whereas the others were negative. 3) Urticaria of unknown genesis. This child was tested for peanut, hazelnut, horse, and mold. The test was positive for horse, discordant for mold, and negative for the remainder. 4) Urticaria of unknown genesis. The test was performed for pollen, cat, dog, and milk. The result was positive for birch, one positive and one negative for timothy, and negative for cat, dog, and milk.

Of the 14 one negative/one positive results, the negative results in eight cases were totally negative and did not even express erythema.

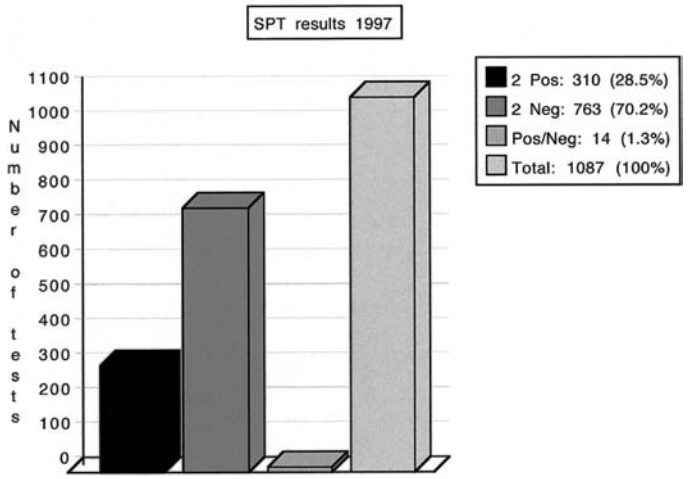

Figure 2. In infants younger than 2 years of age, 340 duplicate tests were performed in 1997. The figure shows the relationship among results of duplicate positive, duplicate negative, and diverging results with one positive and one negative wheal. Three of 340 tests show diverging results, corresponding to 0.9% (95% confidence interval 0.2 to 2.6%).

Table 1. Summary of 14 Patients where Skin Prick Testing with Food and Inhalation Allergens Using the Duplicate Method Showed Diverging Results

	1 pos and 1 neg wheal (0 mm)	1 pos and 1 neg wheal (<3 and 2 mm, resp.)
<2 years	Peas (6.0/0)	Wheat (3.0/2.5)
		Hazelnut (3.5/2.5)
2–18 years	Peanut (4.5/0)	Peanut (4.0/2.5)
	Rabbit (3.0/0)	Egg (3.0/2.0)
	Horse (5.5/0)	Timothy grass (3.5/1.5)
	Horse (4.0/0)	*Mucor racemus* (2.5/1.0)
	Dog (3.5/0)	
	Timothy grass (2.5/0)	
	Timothy grass (4.0/0)	

The first column shows totally diverging results where the negative wheal was 0 mm. The second column shows diverging results with one positive and one negative wheal, using a cutoff limit of 3 and 2 mm, respectively, for food and inhalation allergens. Numbers in brackets are the mean diameter of the wheals.

DISCUSSION

Duplicate skin prick testing has previously been recommended and has been used routinely in our department.[12] However, because of six cases in 1996 to 1998 where infants developed generalized allergic reactions in connection with the test situation, the routine has been altered and, since mid-1998, the infants have been tested with single skin prick tests only.[7] Since the single test was introduced we have only had one case of generalized allergic reactions when performing skin prick test.

Earlier reports that accidental negative tests are common have been the reason for performing the skin prick test in duplicate. Indeed, some authors still recommend duplicate tests.

In our study, however, we found 14 duplicate results with one positive and one negative wheal of the 1,087 tests performed. This corresponds to only 1.3% of the tests. In the youngest age group (<2 years), even fewer, 3 of 340 (0.9%), had an outcome of one positive/one negative test result. In the younger age group, significant hyporeactivity has been previously reported and, therefore, smaller prick test wheals. This suggests that a lower cutoff limit might have to be allowed in this age group for positive skin prick test results. If 2 mm is used as the limit for food allergens in the youngest age group, the outcome of the present study would be only 1 of 340 (0.3%) with one positive/one negative test result.

In children under 2 years of age, tests for food allergens predominate, ie, 89% of all tests performed. Because few standardized food-allergen extracts are available on the market, and those that are available are titrated in weight/volume, which is a very unsatisfactory method of titration, fresh or frozen food specimens are recom-

mended.[9,17,18] The allergen concentration is likely to vary in nonstandardized extracts. For that reason, they are known to be a risk factor for inducing generalized reaction.[18] However, the use of nonstandardized extracts does not seem to increase the test outcomes with diverging results between the wheals, which amounted to only 0.9% in this age group. This finding supports the use of the single prick test.

CONCLUSION

Considering the risk of inducing a summation of the reactions, and thereby a generalized allergic reaction, when applying an extra allergen load on the limited surface of the small arm, we conclude that the results of the present study justify using single prick test, at least in the youngest age group and likely when testing children of all ages.

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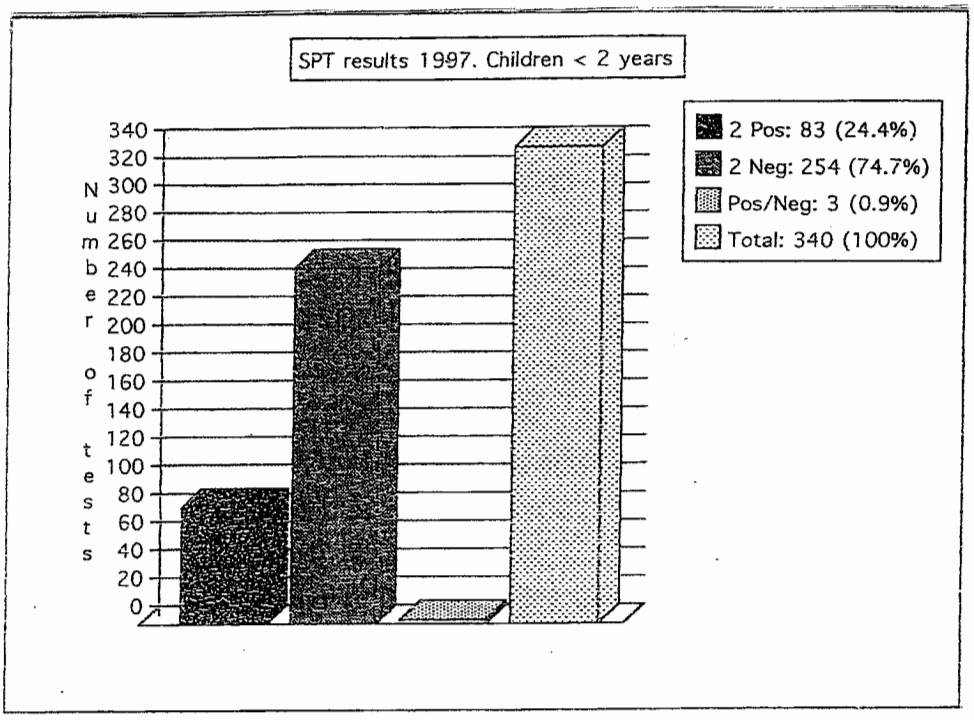
Correction: Paper II "Skin prick test in duplicate – is it necessary?" Fig 2.



In infants < 2 years of age, 340 duplicate tests were performed in 1997. The figure shows the relationship between results of duplicate positive, duplicate negative and diverging results with one positive and one negative wheal. Three of a total of 340 tests show diverging results, corresponding to 0.9% (95% confidence interval 0.2-2.6%).

Paper III

A new model for low-dose food challenge in children with allergy to milk or egg

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Abstract

Aim: Atopic eczema and food allergy are common in early childhood. Children seem to gradually develop tolerance to milk and egg, and it is a relief for families when their child can tolerate small amounts of these basic foods, even if larger doses may still cause symptoms. The aim was to develop a model for low-dose oral food challenge, facilitating re-/introduction of milk or egg.

Method: In 39 children sensitized to milk and/or egg, we performed 52 challenges using a new standardized model for low-dose oral food challenge. The recipes were validated for blinding with sensorial tests.

Results: Four children challenged to milk had a positive challenge outcome. There were no significant differences with respect to family history, associated atopic manifestations, nutritional supply, eczema severity, or skin prick test compared with the non-reacting children, but total and specific IgE values were significantly higher. All but two of the non-reacting children were able to introduce milk and egg into their diet without problems.

Conclusion: We report recipes and a protocol to be used for standardized open and double-blind placebo-controlled low-dose food challenge in young children, enabling the introduction of small amounts of egg and milk into the diet during tolerance development.

Keywords: Atopic eczema, Double-blind, Food allergy, Food challenge, Skin prick test

Introduction

Atopic eczema (1) usually begins early in life and affects more than 10% of infants (2). In children with eczema, 33-37% have food allergy (3, 4). Over time most allergies to egg and milk are outgrown, in about 80 % of children in early childhood (5). Tolerance is likely to develop gradually, which is clinically important, at least in respect of such basic foods as milk and egg, and there is no indication that such development is delayed if the intake of these foods is increased as the child begins to achieve tolerance (6). As avoidance of food allergens is currently the only treatment for food allergy, accurately identifying children with a clinically relevant food allergy is important and will enable the children to avoid unnecessary, and even harmful, dietary limitations (4, 5, 7).

Skin prick test (SPT) is frequently used in evaluating food allergic children. A positive outcome of SPT indicates only sensitization and not necessarily clinical allergy. The test can remain positive long after clinical tolerance is achieved or may represent a short-term reaction in infancy with no correlation with oral challenge test results (4, 6, 8, 9).

Confirming or refuting food allergy often requires oral challenges. An open challenge directed by SPT is a common approach in clinical practice when screening for food allergy. However, in diagnosing clinically relevant allergy, such as in atopic eczema where day-to-day variations play a major role, and always in research situations, the double-blind placebo controlled food challenge (DBPCFC) represents the 'gold standard' (5, 6, 8, 10). The successful performance of oral food challenge in young children requires considerable preparation, ingenuity, and patience (11, 12). Developing new recipes is time-consuming, and correct blinding for the double-blind challenge must be ensured. Children may reject a food because it has a strange taste, and their resistance can even lead to vomiting as an emotional reaction (13). Therefore, natural food should preferably be offered in the way the patient would normally eat it (3, 13). After a negative blind challenge, either an open challenge should be performed or the parents should be instructed to add food to the diet in small but successively increasing amounts. Once the food is tolerated, it can be eaten as often as desired, and in normal portions (14).

Different protocols for the DBPCFC have been used, and a proposal for standardization was recently published (10). However, no protocol has been suggested for low-dose challenge suited to young children outgrowing their food allergy to prove that a child can eat small amounts of the food without incurring an allergic reaction.

Aims

The aim of this prospective study was to develop recipes and a protocol for low-dose oral food challenge to milk and egg to be used for standardized open and DBPCFC in young children outgrowing their food allergy so as to facilitate early re-/introduction of small amounts of milk and egg into the diet.

Methods

Children

The entire study group consisted of 123 children (71 boys, 52 girls) under two years of age participating in a larger prospective study of the clinical and immunological development in small children with eczema and suspected food allergy. The children were recruited to the study between June 1999 and September 2001 after referral to our pediatric clinics from primary-care physicians because of eczema and suspected allergy to milk and/or egg. Language/communication problems and/or complicating diseases were criteria for exclusion.

At the first visit 78 children tested positive for milk and/or egg using the SPT with cut-off limit of $\geq$ 3 mm as recommended in EAACI position papers and performed as described previously (15, 16). They were prescribed a diet minus the corresponding food/foods, and if breastfeeding, the foods were also excluded from the mother's diet. They were given a regime of skin lubrication and steroid topical treatment when required. We enquired about other atopic manifestations, family history, environmental factors, and the nutritional supply. The eczema was evaluated with the Severity Scoring of Atopic Dermatitis (SCORAD) (17), and criteria for atopic eczema were assessed according to Hanifin-Rajka (18). Blood samples were obtained, and total and specific IgE in serum were measured using the UniCAP 100® system, Pharmacia. In 31 children the foods were re-introduced early either by the parents at home, who did not wait for a controlled challenge or did not comply with the elimination diet, or by open challenge after a short elimination period. These children were regarded as food sensitized but not food allergic. The remaining 47 milk and/or egg-allergic children, in whom early food challenge could not be performed due to severe eczema, SPT > 10 mm or recent allergic reactions on accidental exposure to the offending foods, were re-evaluated annually, clinically and with SPT and SCORAD (5, 19). Eight children were excluded from challenge during the study period because of SPT remaining more than 10 mm and/or severe generalized allergic reactions on accidental exposure to the foods within the previous six months (10). The remaining 39 children underwent oral food challenge when SPT were $\leq$ 10 mm and SCORAD were $\leq$ 25.

Recipes

We developed recipes for mixing egg and milk in tasty products suitable for young children to be used in both double-blind and open standardized oral food challenges. To make the recipes acceptable and easy to administer, egg was administered as an ingredient in a sponge cake and milk was added to the milk substitute usually given to the child. When we baked the sponge cake, we found it necessary to heat the milk-free margarine to avoid discoloration. In the recipes the exact amount of supplied milk and egg in each dose are provided (Table 1).

Sensorial tests were conducted as a triangle test to ensure that active and placebo challenges were identical (10, 12). The taste panel, comprising 15 adults and 8 children, none with any known food allergy, was asked to detect a perceivable difference between three samples; one, two, three or none of which contained the active substance. All tests were served cool in medical pots that were coded for each test. The participants were allowed to rinse their mouths with water before and after testing. They were then asked to say which of the samples were the same and whether any differed from the others.

Food challenge

The children were allowed a light breakfast two hours prior to the challenge. Antihistamines had been withdrawn three days previously (9). Topical corticosteroids of low and medium class were allowed if required. The children were equipped with an intravenous access during challenge (10). Blood tests obtained for measuring total and food-specific IgE were analyzed

retrospectively after the challenge was performed. Successively increasing doses of the allergen were given in amounts of 0.1, 0.5, 5.0, 15, and 30 ml for milk and 0.1, 0.5, 1.5, 5, and 10 g for egg. The interval between each dose was 20 min (10). Both a doctor and a nurse were present, and rescue medication was ready to be used in case generalized/severe allergic reactions occurred. The challenge was immediately stopped if objective clinical symptoms arose. The children were observed for two hours after the final dose was administered (10). The food challenges were scored positive if objective clinical allergic reactions such as urticaria, vomiting, wheezing, erythema, exacerbation of eczema or acute rhino-conjunctivitis were observed. Clinical reactions within two hours after the final dose were defined as early, thereafter as late (4, 8). In the double-blind challenges, the interval between the two sessions was two weeks. The nurse contacted the family the following day and one week after the challenge to assess the occurrence of late reactions. In the DBPCFC, the code was broken after these two contacts. If neither early nor late allergic manifestations had appeared, the family was instructed to introduce the food in successively increasing amounts. The child was followed up after three months to assess the introduction of the foods and enquire whether symptoms of allergy had appeared.

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.

Ethics

The Human Research Ethics Committees at the Faculty of Health Sciences, Linköping University, and at the Medical Faculty at Uppsala University approved the study.
Informed consent was obtained from the children's parents.

Results

Clinical assessment
In the entire study group of 123 children, 78 were SPT-positive to foods and 45 were SPT-negative. Of the 78 milk and/or egg sensitized children 47 were assessed as food-allergic. These 47 children with food allergy constitute 38% of the 123 with eczema, which concurs well with previously presented figures (3,4). In 39 of the 47 children we were able to proceed with the model for low-dose challenge.
These 39 children were 5 months (median, range 1-23 months) at the first visit; 18 were girls and 21, boys. Symptoms of allergy other than eczema were: urticaria (n=13), gastrointestinal symptoms (n=13), allergic rhino-conjunctivitis (n=10), and asthma/wheezing (n=7). In 11 children eczema was the only symptom. In 6 children the SCORAD was 0 points. These 6 children had all expressed eczema on referral but had successfully treated the eczema before the visit to our clinic. Twelve children had three or more manifestations of atopic disease. All but 4 had a positive family history of atopy (first-degree relative). Thirty-seven children were breastfed for 7 months (median, range 2-20 months). For the first six months of life they were either exclusively breast-fed or received a documented hypoallergenic formula (Nutramigen®). All 39 children fulfilled the criteria for atopic eczema according to Hanifin-Rajka and expressed SPT positivity to milk and/or egg. We obtained blood tests in 36 children for measuring total and specific IgE.
The children were consecutively re-evaluated. When SPT was $\leq$ 10 mm and SCORAD points were $\leq$ 25, the 39 children underwent oral food challenges. A total of 52 food challenges were performed: 23 to milk and 29 to egg, (30 double-blind placebo-controlled, and 22 open standardized). The age at the challenge procedure was 39 months (median, range 18-66 months)**.** We obtained blood samples in 44 of the challenges to analyze total and specific IgE.
The results of SPT, SCORAD, and total and specific IgE from the first visit and when challenged are summarized in Table 2.

Recipes and outcome of the *sensorial tests*
The recipes and the protocol for standardized open and DBPCFC were well accepted by the children. The sensorial tests performed by the taste panel did not show any difference in taste or appearance in the samples with or without the active substance.

Outcome of food challenge
In 4/52 challenges the results were positive, with immediate allergic symptoms. All 4 children had a family history of allergy. They were subjected to DBPCFC to milk and reacted on the first to third dose administered. One child had a negative SPT at the time of the challenge but expressed an SPT of 10.5 mm when re-tested two weeks after the challenge. This child had the highest value of the four for milk-specific IgE, 4.34 kU_A/l. All received adequate treatment with emergency medication and recovered well. The four cases are described in detail in Table 3. There was no significant relationship between the size of the SPT and the challenge outcome or in SCORAD points and challenge outcome. There was, however, a significant relationship between the levels of both total and food-specific IgE and challenge outcome, $p<.005$ and $p<.01$, respectively.

Post food challenge follow-up
At the three-month follow-up, three of the four children with a positive challenge outcome were still on a milk- and egg-free diet. One child (child 1, tab.3) had, at its parents' initiative, received small amounts of milk in its diet without problems. All but two of the non-reacting

infants had successfully introduced the food into their diet without reactions. One family had chosen not to introduce milk in spite of a negative challenge because the twin brother reacted strongly to milk at challenge, and it would be difficult to have the two boys on different diets. One of the children with a negative challenge outcome displayed gastrointestinal symptoms such as flatulence and loose stools when milk was introduced. Therefore the milk was withdrawn from the diet. The child's symptoms were interpreted as non-IgE-mediated hypersensitivity (skin prick test 0 mm and specific IgE < 0.35 kU_A/l when challenged), and a trial period with lactose-free diet was recommended to assess a possible lactose intolerance.

Discussion

In this study we have developed recipes and a protocol for low-dose oral food challenge. The recipes were tested as open standardized and DBPCFC, and both were tolerated well. 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 when using unnatural forms of foods. Our aim was to rule out children who might have an acute allergic reaction when exposed to small amounts of the food, and to identify the children for whom it would be possible to start introducing milk and egg in small, but successively increasing amounts.

The size of the SPT and the concentration of IgE are commonly used tools to direct when to perform challenge. Opinions differ concerning when SPT conclusively predicts a positive challenge. The traditional cut-off level of $\geq$ 3 mm or greater has a great potential for diagnostic error (20), and different cut-off values have been proposed in several studies, varying from 5 to 12.5 mm for milk and from 4 to 13 mm for egg (5,21,22). Cutaneous reactivity might, however, vary with age, time of day, season, and the patient's gender. Therefore, different cut-off values are likely to be required for different subpopulations of children (5, 6, 20). Thus, when contemplating a challenge, SPT size and IgE values may be useful tools, but it is more important to give reasonable consideration to the severity of previous reactions and the kind of allergen involved (6). In the present study in children sensitized to egg and milk, we set the cut-off level of SPT to weal sizes $\leq$10 mm. Four children who were SPT positive to milk had a weal size of >8 mm; 3 of them had a positive challenge outcome. In children challenged to egg, 13 had an SPT weal size of > 7 mm and none of them reacted at challenge.

The 4 children who had a positive outcome of the challenge reacted to very small amounts of the allergen. All 4 underwent a double-blind challenge to milk. There were no significant differences with respect to family history, associated atopic manifestations, nutritional supply, SCORAD points, or SPT positivity compared with the non-reacting children, but total and specific IgE the values were significantly higher. As results from one population do not necessarily apply to children in another population, and the study group was limited to 39 children, the results should be interpreted with caution. In some of the challenges, the children might already have achieved full tolerance and in others, tolerance development was ongoing. Our model does not distinguish between these conditions.

For the non-reacting children, the food in question was introduced without problems in all but two cases: one child due to the difficulties of having two separate diets for twin brothers, and the other because of suspected non-IgE-mediated hypersensitivity. In some of the cases, the parents reported episodes with somewhat aggravated eczema at follow-up. These resolved themselves spontaneously, however, and the introduction of the food was carried on without interruption. Fluctuations in the severity of eczema are part of the nature of the symptoms and were interpreted as having no connection with the introduction of the foods (15).

Because tolerance is likely to develop gradually, parents may notice long before the child tolerates normal amounts of milk and egg that an intake of small amounts of milk and egg in cooked food no longer produces any symptoms. It is a relief for families when their child can tolerate small amounts of milk and egg in bread or cooked food, even if larger doses may still cause symptoms (6). Parents are spared the constant fear that the child might have a severe allergic reaction if accidentally exposed to small amounts of the food, which benefits the child's quality of life as well as its nutrition and growth (7, 23). The model for low-dose challenge used in this study can facilitate the re-/introduction of milk and egg in young sensitized children outgrowing their allergy. It is, however, important to inform the parents of the amount of the food the child has been tested with and that a negative outcome when challenged with cooked egg does not exclude a possible reaction to raw egg. We instructed

the parents to start with the amount used in the challenge as the daily intake and only successively to increase the amounts. Support from the allergy nurse via regular telephone contact encouraged the parents to continue introducing the foods at home and successively reach an age-estimated daily intake. The introduction of the foods at home following a negative challenge under the telephone supervision of an experienced allergy nurse may replace the regular open challenge after a negative double-blind challenge.
Performing food challenges is, however, never without risks. The size of the SPT weal and specific IgE levels in sera are not predictive of how severe the reaction will be when the outcome of food challenge is positive (3, 10, 22). As the reactions can be severe, adequate equipment for immediate treatment and the presence of trained staff are always necessary. Moreover, even if the risk of a severe reaction is remote, the challenge should take place in a hospital setting (9, 10, 13).

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Table 1.

Recipes for low-dose oral food challenge to egg and milk

A. Sponge cake for egg challenge.
The cake is used for open challenge (version 1) and double-blind challenge with egg (version 1 + 2). In both cases, the cake is prepared without milk or wheat.

1. With eggs.
Crack 3 eggs, and weigh the raw eggs.
Mix: 2.6 dl (180 g) wheat-free baking flour
(we used Semper Fin Mix®, based on rice, potatoes and maize)
with 2 dl white sugar + 10 ml baking soda + 15 ml vanilla sugar
Add the eggs, 2 dl water, and 50 g warm melted milk-free margarine
Bake in oven for 40 min at 175° C.
After the cake has cooled: weigh the whole baked cake.

2. Without eggs.
Mix: 2.6 dl (180 g) wheat-free baking flour
(we used Semper Fin Mix®, based on rice, potatoes and maize)
with 2 dl white sugar + 10 ml baking soda + 15 ml vanilla sugar
Add 2 dl water, 5 ml molasses and 50 g warm melted milk-free margarine
Bake in oven for 40 min at 175° C.
After the cake has cooled: weigh the whole baked cake.

Preparation of the samples:

1. *Cake with eggs.*
Divide the weight of the cake by the weight of the eggs. Multiply the quotient by 0.1, 0.5, 1.5, 5.0 and 10, respectively, to get the five required amounts in grams for the challenge.
Prepare the portions by using a letter scale or equivalent high-precision scale.
Dispense the portions in separate plastic pots, and number-code them I-V.

2. *Cake without eggs.*
Take the same weights in grams as those used for the cake with eggs for each portion and dispense them in plastic pots. Number-code the pots I-V.

B. Milk mixtures for milk challenge.
For masking, we used the milk substitute usually taken by the child. The mixture is used for open challenge with milk (version 1) and double-blind challenge with milk (version 1 + 2).

1. With milk.
Mix 0.1, 0.5, 5.0, 15 and 30 ml, respectively, of raw low-fat milk with equal amounts of the child's milk substitute to get the five required amounts for the challenge.
Dispense the portions in separate plastic pots, and number-code them I-V.

2. Without milk.
Dispense 0.2, 1.0, 10.0, 30 and 60 ml of the childs ordinary milk substitute in separate plastic posts and number-code them I-V.

Table 2.

	Age	SCORAD	SPT milk	SPT egg	Total IgE	Spec IgE milk	Spec IgE egg
	months	points	mm	mm	kU/l	kU_A/l	kU_A/l
	n=39	n=39	n=25	n=36	n=36	n=36	n=36
At first visit	5 (1-23)	26.4 (0-77)	6.0 (3-11)	7.2 (4-16)	42.4 (1-309)	0.46 (<0.35-23.4)	1.22 (<0.35-38.4)

	Age	SCORAD	SPT milk	SPT egg	Total IgE	Spec IgE milk	Spec IgE egg
	months	points	mm	mm	kU/l	kU_A/l	kU_A/l
	n=15	n=15	n=15	n=15	n=13	n=13	n=13
DBPCFC to milk	36 (21-66)	1.0 (0-25)	0 (0-10)	6 (0-20)	39.1 (9.6-552)	<0.35 (<0.35-4.34)	<0.35 (<0.35-11.5)

	Age	SCORAD	SPT milk	SPT egg	Total IgE	Spec IgE milk	Spec IgE egg
	months	points	mm	mm	kU/l	kU_A/l	kU_A/l
	n=15	n=15	n=15	n=15	n=11	n=11	n=11
DBPCFC to egg	41 (34-57)	3.5 (0-12)	0 (0-6)	6.5 (0-10)	26.1 (7-146)	<0.35 (<0.35-<0.35)	<0.35 (<0.35-44.4)

	Age	SCORAD	SPT milk	SPT egg	Total IgE	Spec IgE milk	Spec IgE egg
	months	points	mm	mm	kU/l	kU_A/l	kU_A/l
	n=8	n=8	n=8	n=8	n=8	n= 8	n=8
Open FC to milk	34 (18-40)	5.5 (0-25)	0 (0-9)	7 (0-11)	65.1 (10.5-113)	<0.35 (<0.35-0.58)	<0.35 (<0.35-4.91)

	Age	SCORAD	SPT milk	SPT egg	Total IgE	Spec IgE milk	Spec IgE egg
	months	points	mm	mm	kU/l	kU_A/l	kU_A/l
	n=14	n=14	n=14	n=14	n=12	n=12	n=12
Open FC to egg	44 (38-61)	3.5 (0-20)	0 (0-8)	5.5 (0-10)	50.0 (8.6-895)	<0.35 (<0.35-<0.35)	<0.35 (<0.35-1.22)

Characteristics of the 39 children undergoing challenges. Results of SCORAD, SPT, total and specific IgE at first visit and at the time of the challenge. All values are expressed in median (range).
DBPCFC = Double-blind placebo controlled food challenge, Open FC= Open food challenge

Table 3.

	Age months	SCORAD points	SPT mm	Total IgE kU/l	Spec IgE milk kU_A/l
Child 1 (girl)	66	0	10	226	<0.35
Child 2 (boy)	40	0	8	552	2.5
Child 3 (boy)	39	10.1	9	232	2.0
Child 4 (boy)	21	0.4	0	321	4.34

The four children with positive outcome of challenge. They were all subjected to DBPCFC to milk and reacted to the first to third dose administered. The children's age and the values of SCORAD, SPT, and total and specific IgE on the day of the challenge.

Paper IV

Nitric oxide urinary products in infants with eczema

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Abstract

*Background***:** Eczema is characterized by inflammation of the skin and is commonly induced by food allergy. It has been suggested that nitric oxide (NO) plays a role in eczema, food allergy and intestinal inflammation.

Objective: The purpose of this study was to evaluate the urinary levels of NO breakdown products in children with eczema and to assess the effect of eczema treatment on clinical symptoms and on the NO levels.

Method: Ninety-four infants with eczema were examined twice, with an interval of 6 weeks. The sum of nitrite and nitrate was measured with a colorimetric method in urinary samples from both visits, and the results were compared with clinical data concerning eczema severity, nutrition, gastrointestinal symptoms, asthma, and skin prick sensitivity.

Results: The levels of NO products increased significantly from the first to the second examination, in parallel with a significant improvement in the eczema, both in children on an elimination diet and children treated only with skin care. There was no correlation between eczema severity and urinary NO levels.

Conclusion: On treatment by elimination diet and/or skin care, the eczema significantly improved. Levels of urinary NO breakdown products increased significantly during the same period. We propose that this could be due to a CD4+ T helper 2 /T helper 1 shift induced by the eczema treatment. Individual variations in NO-induced-feedback downregulation of T helper1 and T helper 2 proliferation can explain the variations in NO response.

Key words: children, eczema, nitric oxide, Th1/Th2 shift

Introduction

Eczema is a common health problem affecting 10-20% of children worldwide (1,2). It is characterized by inflammation of the skin and is induced by food allergy in approximately 35% (3). Altered intestinal permeability with increased absorption of large molecules, but normal uptake of low-molecular weight species, has been reported both in eczema and food allergy, indicating inflammation of the intestinal mucosa (4). Change in permeability, with altered absorption of polyethylenglycols (PEG 400 and PEG 1000), has also been demonstrated after cow's milk challenge in milk-allergic children. Modulation by pre-treatment with sodium cromoglycate showed that an anti-inflammatory drug reversed the reaction and the effect of the challenge (5). Moreover, increased gut permeability has been demonstrated in asthmatic children, suggesting that the entire mucosal system may be affected in allergic disease (6).

Nitric oxide (NO) is a multipotent intracellular messenger modulating various physiological processes, including blood vessel dilatation and immune function, and can be produced by almost all mammalian cells. NO reacts rapidly with oxygen, yielding nitrite and nitrate. NO is formed from L-arginine by NO synthase isoforms (NOS). There are two Ca^{2+} dependent constitutive forms (cNOS), eNOS and nNOS, and one Ca^{2+} independent inducible form, iNOS. The constitutive form, which produces low amounts of NO, has generally been associated with the regulating of the homeostatic function, whereas iNOS, which produces large amounts of NO and is induced in various cells by inflammatory stimuli, such as endotoxins and different cytokines, has been associated with severe tissue damage (7).

iNOS has also been shown to have multiple positive biological effects. It is essential for normal healing of the skin and intestinal mucosa, kills certain bacteria, may be important in regulating T cell proliferation and the differentiation T helper (Th)1 versus Th2, and may regulate leukocyte recruitment. Because all this may counter the effect of the toxic metabolites also produced by iNOS, it would be too simplistic to regard iNOS as only harmful (7). As there may be as many as 15 different types of cells that can express iNOS, it would also be inappropriate to assume that iNOS functions the same way in each cell. The fact that NO can produce toxic metabolites applies to a subset of cells, including oxidant-producing cells such as neutrophils, which, unlike epithelial cells, can produce large amounts of peroxynitrate (7). Significant quantities of eNOS and nNOS are found in the digestive tract under normal conditions, as are small amounts of iNOS. Inhibition of NO has been shown to cause the features of intestinal inflammation, including neutrophil recruitment, increased oxidative stress, mast cell degranulation, and increased microvascular and epithelial permeability (7). For clinical use, the possibility of measuring the levels of NO in exhaled air to assess inflammation in the airways has become an adjunct in monitoring treatment (8, 9).

Excessive NO production has been suggested to contribute to mucosal damage, therefore urinary analysis of the breakdown products of nitric oxide is a method that has been used in children with celiac disease (CD) (10,11). Unlike NO, nitrite and nitrate are stable breakdown products that can be measured both in serum (12) and urine (10,11). For children, urinary sampling is more convenient than blood sampling. Children with untreated CD, i.e. with small intestinal mucosa characterized by crypthyperplastic villous atrophy and severe inflammation, displayed significantly higher levels of nitric oxide products, reflecting the inflammatory response, than did children on a gluten-free diet with normalized mucosa. There was also a good correlation between the CD severity and the level of NO urinary products (10,11).

Moreover, a study of children with eczema demonstrated higher levels of serum nitrate than among healthy children and lower nitrate levels after eczema treatment. The serum nitrate

levels also correlated with the severity of the eczema (12). Apart from this study (12), there is little information available regarding the levels of nitrite/nitrate in eczema.

The aim of this study was to assess the levels of NO breakdown products in urine in children with eczema, with and without sensitization to food allergens, and to compare the values before and after treatment of eczema.

Materials and Methods

The study group consisted of 94 children (58 boys, 36 girls) under two years of age participating in a prospective study of the clinical and immunological development in small children with eczema and suspected food allergy. The children were recruited to the study on referral to our pediatric clinics from primary-care physicians. Language/communication problems and/or complicating diseases were criteria for exclusion. The diagnosis of eczema was established using the criteria defined by Hanifin and Rajka (13). The extent and severity of eczema were assessed with the Severity Scoring of Atopic Dermatitis (SCORAD) method (14), taking into account both the extent and severity of eczema as well as the consequences of the skin disorder (degree of pruritus and sleeping disorder assessed by the parents). According to this classification, children were judged to have mild (SCORAD $\leq$25 points), moderate (SCORAD 26 - $\leq$ 50 points), or severe (SCORAD >50 points) eczema. The SPT and the SCORAD assessment were performed by experienced allergy research nurses. Before the start of the study, the nurses practised scoring on children with eczema to reduce inter-observer variability. SPTs were performed to cow's milk and egg, the main food allergens in Swedish children of this age group. On positive outcome, the parents were instructed by a dietician to eliminate the offending allergen from the child's diet and if breastfeeding also from the mothers diet. For the first six months of life they were either exclusively breast-fed or received a documented hypoallergenic formula (Nutramigen®).The parents received instructions and were given a practical demonstration of how to treat eczema and dry skin. Emollients for skin care were provided as well as anti-inflammatory treatment with topical glucocorticoids if needed. A morning sample of urine was obtained. After 6-8 weeks, at a second visit, the SCORAD assessment was repeated, and another urinary sample was collected. The children's diet, according to Swedish recommendations for infants, did not contain food items with high levels of nitrite/nitrate, such as several vegetables (e.g. spinach, beetroot, rhubarb, fennel, celery) and smoked and salted meat products. The criteria used for asthma were any episode of wheezing if related to exposure to allergens or combined with atopic eczema, or at least three episodes of wheezing in the absence of atopy and exposure to allergens. The children's age at the first visit was 7.5 ± 5.2 months (mean ± SD) and at the second visit, 10 ± 5.4 months (mean $\pm$ SD).

Methods

In the urinary sample, the sum of nitrite and nitrate was measured as an indirect indicator of the NO production (15). In short, the nitrite content was measured with a colorimetric method based on Griess reaction for nitrite. In a PBS-diluted sample, nitrate was converted using nitrate reductase from Aspergillus (16). Next, 50 µl of the diluted urine was mixed with 10 µl NADPH (1 µM) followed by 40 µl containing nitrate reductase (80 U/l, Roche, Basel, Switzerland), glucose-6-phosphate (500 µM) 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 µl sulfanilamide (1% in 5% phosphoric acid) and 100 µl naphtylethylenediamine (0.1 %). The resultant color was read with a spectrophotometer (Vmax, Molecular Devices, Sunnyvale, CA) at 540 nm.

SPT

SPT was performed as prick-prick test as described previously (17). In accordance with the EAACI position paper, the results were considered positive when the mean diameter (half of the sum of the largest diameter and its perpendicular) of the wheal was $\geq$ 3 mm greater than the negative control (18).

Statistics
For statistical evaluation, Student's t-test in the statistical software program SPSS 11 for Mac 0SX was used.

Ethics
The study was approved by the Human Research Ethics Committee at the Faculty of Health Science in Linköping and at the Medical Faculty at Uppsala University. Informed consent was obtained from the children's parents.

Results

Skin prick test positivity
In 62 children, the SPTs to egg and/or milk were positive, whereas 32 children displayed negative SPTs.

Eczema assessments
The majority of the children, 80/94 (85%), fulfilled the Hanifin-Rajka criteria for AE. The SCORAD value for the whole group was 22.0 ± 6.0; 17.1; 0-77 (mean ± SD; median; range) at the first visit, and 11.6 ± 10.4; 9.0; 0-45.2 at the second visit ($P<0.001$ for both paired and unpaired test). For the SPT-positive children the values were 24.2 ± 17.8; 19.9; 0-77 at the first visit, and 11.7 ± 10.8; 9.1; 0-45.2 at the second ($P<0.001$ for both paired and unpaired test). The corresponding figures for the SPT-negative group were 17.8 ± 14.5; 15.2; 0-50.8 at the first, and 11.2 ± 8.6; 9.0; 0-22.2 at the second visit ($P<0.05$ unpaired, and $P<0.005$ paired).

Nitrite/nitrate in urine on the first and second occasions
The values for the whole group were 420 ± 428; 286; 65-3174 μM (mean ± SD; median; range) on the first, and 711 ± 775; 448; 36-4648 μM on the second occasion ($P<0.005$ paired, and $P<0.002$ unpaired test). In SPT-positive children, the levels were 436 ± 497; 238; 65-3174 μM, on the first, and 646 ± 705; 407; 68-4648 μM on the second occasion (ns). For SPT-negative children, the respective values were 387 ± 249; 316; 102-903 μM, and 840 ± 893; 530; 36-3680 μM ($P<0.01$ for both paired and unpaired test). There was no significant difference between the SPT-positive and SPT-negative group at the first or second visit. Values of nitrite/nitrate are shown in Fig 1. For the majority of children, 60/94, the values at the second measurement were higher than at the first. The change over time in 10 children with a value above 1406 (mean + 2 SEM in healthy reference children (10)) is displayed in Fig 2. These 10 children did not differ from the others when the severity of eczema was compared at the second visit.

Comparing urinary nitrite/nitrate levels with age, atopic symptoms, eczema severity and nutrition
Nitrite/nitrate levels and SCORAD value were not correlated (Fig 3). When examined at the first visit, 52/94 children were still being breastfed, whereas 91/94 had been breastfed from birth until at least 2 months of age. Breastfeeding did not correlate with nitrite/nitrate values. Nor were nitrite/nitrate values influenced by age, presence of gastrointestinal symptoms, or airway symptoms.

Discussion

As expected, the treatment with skin care and/or food interventions improved the eczema, as assessed by decreased eczema scoring. However, contrary to our expectations, urinary NO breakdown products increased significantly in parallel with the eczema improvement, and there was no correlation between eczema severity and urinary nitrite/nitrate levels. This is in contrast to a previous study of children with eczema. They 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 (12). However, that study was performed with serum samples, and the children were older (mean age 2.2 years). This presumably means that their eczema is of a more chronic nature. No information was provided regarding diet, i.e. intake of food rich in nitrite/nitrate, or sensitizations to foods requiring elimination diets.
In this study, urinary excretion of NO products increased significantly after treatment, from 420 ± 428 µM (mean ± SD) on the first occasion to 711 ± 775 µM at the second investigation. A possible explanation for increasing levels of nitrite/nitrate would be large consumption of food products containing nitrite/nitrate. This is not a likely explanation, as parents in Sweden are advised not to serve these food items to their infants. Another source of NO production in the body might be untreated asthma, as several studies show the usefulness of analyzing exhaled NO products as a sign of airway inflammation. Among the 94 children in this study, 20 were diagnosed with asthma, and their urinary nitrite/nitrate levels did not differ from those of children without airway problems.

Previous studies of urinary breakdown products by our research group have focused on children with celiac disease (CD). Children with untreated CD have displayed very high nitrite/nitrate values, 4147 ± 1102 µM (mean ± SEM, n=20)(10). During gluten exposure, increased expression of iNOS was demonstrated in small intestinal biopsy samples (19), which made increased urinary excretion of nitrite/nitrate in this condition a reasonable finding. After treatment with a gluten-free diet, i.e. when healing of the intestinal mucosa has been demonstrated in small intestinal biopsy, the nitrite/nitrate levels were 1078 ± 1084 µM (mean ± SD, n=25) (11). The levels in healthy children of the same age at 1174 ± 116 µM (mean ± SEM, n=53) (10). In comparison, the levels of nitrite/nitrate in the children with eczema in this study were much lower, especially before treatment, 420 ± 428 µM (mean ± SD). Also at the second sampling after eczema treatment, the levels in this study were lower, 711 ± 775 µM (mean ± SD), but were approaching the levels in healthy children and children with CD on gluten-free diet.
An inflammatory reaction, as in eczema, is thought to result in an activation of the stress system, which induces a Th1/Th2 shift, to provide protection from systemic "overshooting" with Th1-induced proinflammatory cytokines and elevated levels of toxic NO products (20). However, the inhibition of NO may cause increased intestinal inflammation with mast cell degranulation and increased permeability (7), and could reduce the possible positive effects of NO, which may be important for skin and intestinal mucosa healing (7, 20).
Further, the immune modulating effects of NO have recently been studied in asthma by in vitro studies of human bronchial epithelial cells (21). It is generally assumed that NO only has a harmful influence in asthma, by selective downregulation of Th1 responses. However, the cited study showed that NO can limit in vitro expansion of both CD4+ Th1 and Th2 cells and reverse the Th2 to Th1 shift that follows on treatment of inflammation (21). Human epithelial cells upregulate NOS, which causes NO release, which in turn inhibits Th1 and Th2 proliferation. When NOS is specifically blocked, the T cell proliferation is shown to be completely restored. By this feedback loop, the organism may putatively protect the airways

from overwhelming inflammatory response after allergen exposure (21). Interestingly, the authors suggest an individual variation in the efficiency of this feedback loop, explaining the fact that only some of the children sensitized to aeroallergens develop asthma (21).
We hypothesize that our findings with low levels of NO in children with active eczema and increased levels after treatment might be explained by a similar mechanism with upregulation of iNOS, as observed in human epithelial cells in asthma. The majority of the children in our study displayed elevated NO levels after treatment, but not all, which might be explained by individual variations in the feedback system as previously suggested (21). Further investigations of these children at a higher age will reveal if these individual variations persist.

In conclusion, contrary to our expectations we found that levels of urinary NO breakdown products increased significantly after treatment of eczema in parallel with improvement of the skin. The increase might be due to a Th2/Th1 shift induced by the eczema treatment, i.e. by amelioration of the inflammation present in the eczematous skin. Individual variations in NO-induced-feedback downregulation of T helper1 and T helper 2 proliferation might explain the variations in NO response.

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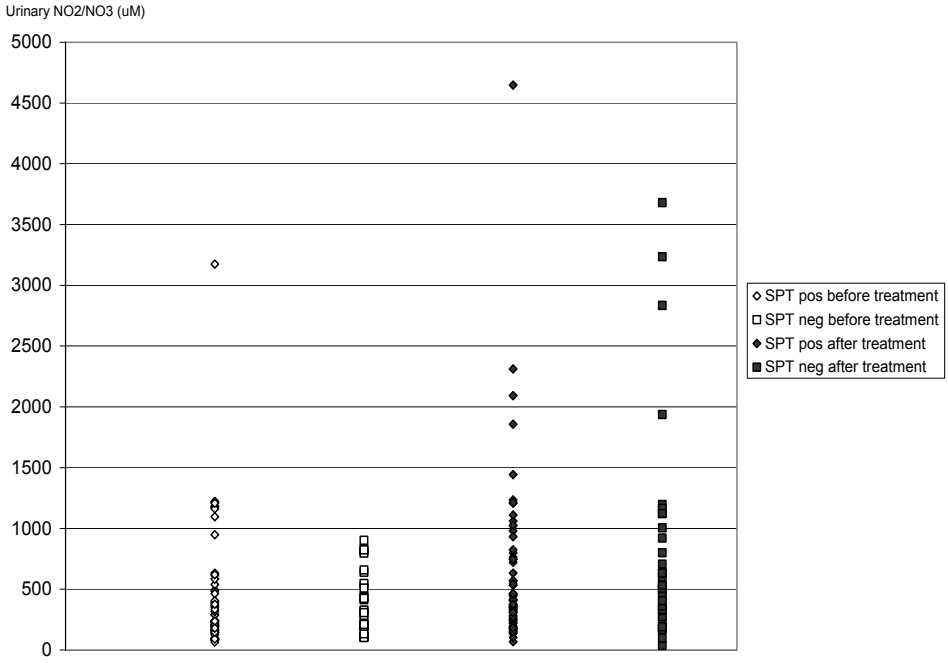
Fig 1.

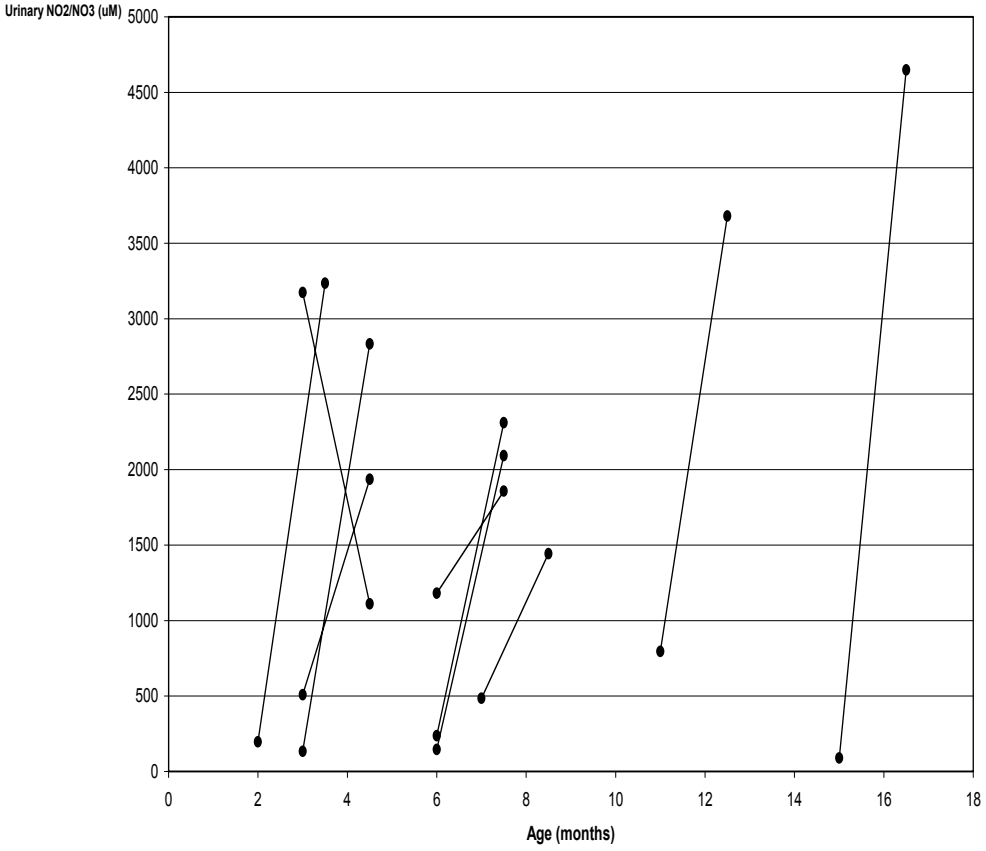
Values of nitric oxide breakdown products in children with eczema



The values of nitrite/nitrate were significantly higher after treatment, 711 ± 775; 448; 36-4648 μM (mean ± SD; median; range), compared with before, 420 ± 428; 286; 65-3174 μM ($P<0.005$).
SPT pos = skin prick test positive, SPT neg = skin prick test negative,

Fig 2.

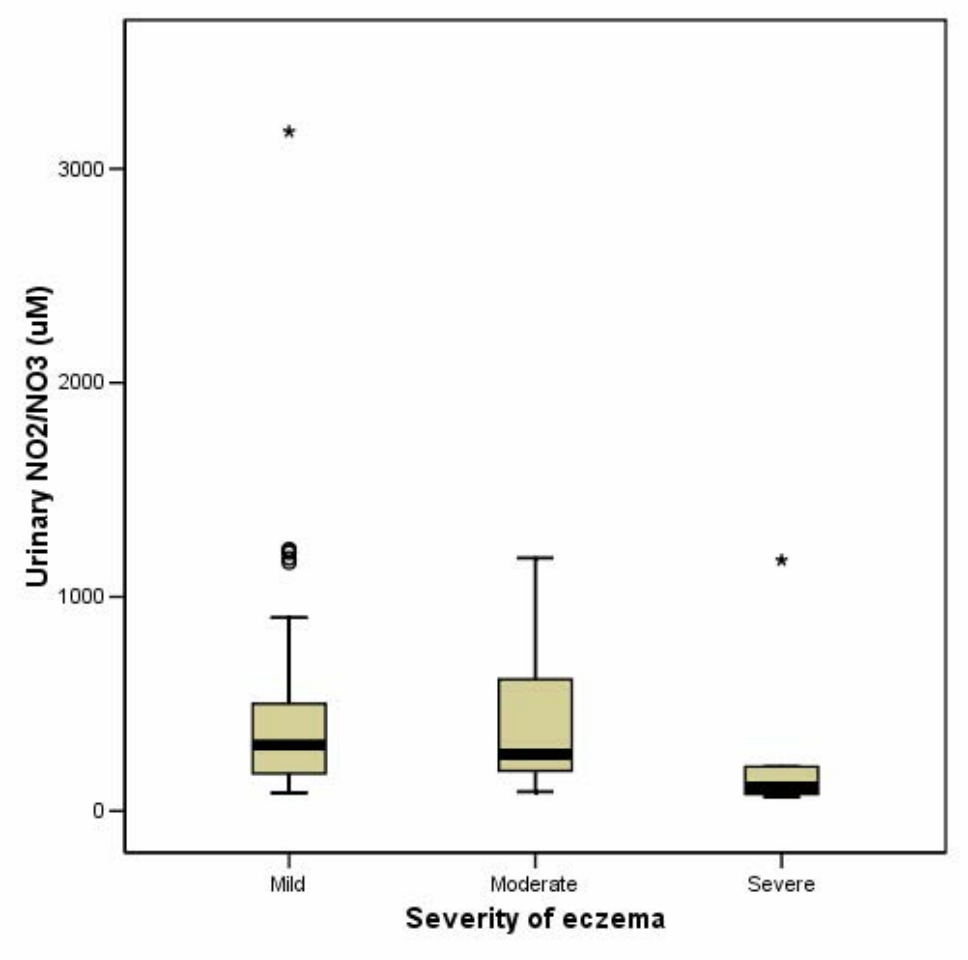
Individual changes in nitrite/nitrate values in urine



The change over time in nitrite/nitrate levels in urine, before and after eczema treatment, in the10 children with a value above 1406 µM (mean +2 SEM in healthy reference children (10)).

Fig 3.

Nitrite/nitrate levels in urine compared with the severity of eczema



The nitrite/nitrate values in urine in the 94 children were not correlated with the SCORAD (Severity Scoring of Atopic Dermatitis) values.

Paper V

Eczema in infancy and the atopic march

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Abstract

Background: Most children with eczema and food allergy will develop sensitization to aeroallergens. Early identification of atopic eczema may provide opportunities to prevent later development of allergic airway symptoms.

Objective: To evaluate children with eczema, with and without sensitization to foods, from referral at < 2 years until follow-up at 4½ years of age.

Methods: We followed 123 children with eczema, 78 sensitized and 45 not sensitized to food, with respect to eczema severity, other allergic manifestations, development of airway sensitization and achievement of food tolerance.

Results: The difference in severity of eczema at referral was significant when comparing children sensitized to milk and/or egg with non-sensitized children, $p<.001$. At follow-up, milk- and egg-allergic children had achieved tolerance in 81% and 68%, respectively. Sensitization to aeroallergens was observed in 58% of the food-sensitized children and 26% of the non-food-sensitized children, $p<.001$. However, the difference in airway symptoms was not significant, 35% and 23%, respectively.

Conclusions: We found that the prognosis for achieving clinical tolerance was very good in children early sensitized and allergic to milk and egg. In all children the eczema improved, with the highest remission rate in non-sensitized children. Children sensitized to foods became significantly more often sensitized to aeroallergens. Low exposure to tobacco smoke may have contributed to the low rate of airway symptoms in the sensitized children at follow-up. However, the sensitization to aeroallergens suggests that the atopic march will continue and the children might subsequently develop allergic airway disease.

Key words: asthma, allergic rhinitis, atopic eczema, food allergy, skin prick test

Introduction

The prevalence of atopic eczema (AE) has increased two- to three-fold during the past three decades (1). The disease affects 10-20% of children worldwide and commonly presents during early childhood (1, 2). The prevalence of food allergy in the first years of life is 6-8 %, and later in childhood 1-2 % (2, 3). In children with eczema, 33-37% have food allergy (4-6), with the most common offending foods being egg and cow's milk.
In this study the terminology proposed by the EAACI nomenclature task force is used. The term *eczema* replaces the former term 'atopic eczema/dermatitis syndrome' (AEDS), and *atopic eczema* (AE) means eczema in a person of the atopic constitution (7).

Natural course of food allergy and AE
The "atopic march" refers to the natural history of atopic manifestations, characterized by a typical sequence of IgE antibody responses and clinical symptoms, which appear during a certain age period, persist over years and decades, and often show a tendency for spontaneous remission with age (8). Within the atopic march, AE and food allergy are often the first manifestations and present individuals destined to a lifetime of allergy and asthma (1). In these children, most allergies to egg and milk are outgrown in early childhood (9, 10). The loss of food allergy is, however, a variable process, depending on both the individual child and the specific food allergy. For instance, whereas most milk and egg allergy is outgrown before school age, allergies to peanuts and tree nuts are rarely lost (3). Following the atopic march, approximately 80 % of children with AE will eventually develop asthma and/or allergic rhinitis (AR), with many outgrowing their AE with the onset of respiratory allergy (1, 11). Early identification of children with food allergy and AE may provide opportunities to prevent the development of asthma (2, 3, 12). Tests for food allergy are recommended in children with eczema without spontaneous improvement in the summer period or without sufficient effect of topical steroids and/or when food allergy is suspected (2). IgE-mediated food allergies are detected by positive skin prick test (SPT) results and/or elevated food-specific IgE-antibodies in serum. Tests showing increased IgE antibody response to milk and egg in infancy are to be considered markers of atopic reactivity in general and may be predictors of subsequent sensitization to aeroallergens (2, 8).

Aims
The aim of this prospective study of children with eczema, with and without sensitization to foods, was to evaluate the course of the disease from referral at < 2 years of age until follow-up at 4½ years of age. We wanted to study what differs between children with eczema who are early sensitized to milk/egg and children with eczema but without sensitization, in respect of heredity, severity of eczema, other allergic manifestations, development of sensitization to airborne allergens, and achievement of food tolerance over these first years of life.

Method

The study population consisted of 123 children (71 boys, 52 girls) under two years of age on referral from primary-care physicians because of eczema and suspected food allergy. The children were recruited to the study over a two-year period, from June 1999 to September 2001. Criteria for exclusion were language/communication problems and/or complicating diseases. The children were tested for sensitization to milk and/or egg (the main food allergens in Swedish children of this age group) using the SPT with a cut-off limit of ≥3 mm, as recommended in EAACI position papers, and performed as described previously (13,14). On positive SPT outcome, the parents were instructed by a dietician to eliminate the offending allergen, and if breastfeeding, the foods were also excluded from the mother's diet. For the first six months of life they were either exclusively breast-fed or received a documented hypoallergenic formula (Nutramigen®).
The diagnostic criteria proposed by Hanifin and Rajka were used to diagnose AE (15). According to which at least three of the four basic features (pruritus, typical morphology and distribution, chronic or relapsing course, and positive family history of atopy) and three or more associated features, such as: early age of onset, xerosis, Dennie-Morgan infraorbital folds, orbital darkening, facial erythema, cheilitis, food intolerance, positive SPT and/or raised serum IgE concentrations, and the influence on the eczema by environmental and emotional factors are required. The severity of the eczema was evaluated with the Severity Scoring of Atopic Dermatitis (SCORAD) instrument (16). The SPT and the SCORAD assessment were performed by experienced allergy research nurses. Before the start of the study, the nurses practised scoring on children with eczema to reduce inter-observer variability. The parents received instructions and were given a practical demonstration of how to treat eczema and dry skin. Emollients for skin care were provided as well as anti-inflammatory treatment with topical glucocorticoids if needed.
We enquired about other atopic manifestations, family history, environmental factors, and the nutritional supply. The criteria used for asthma were any episode of wheezing if related to exposure to allergens or combined with AE, or at least three episodes of wheezing in the absence of atopy and exposure to allergens. The criterion used for AR was rhinitis at least twice after exposure to allergens and not related to infection. For gastrointestinal allergy the criterion was vomiting and/or diarrhea at least twice after intake of an offending food.

The children were re-evaluated at regular intervals clinically, and with SCORAD; the SPT-positive children also with repeated SPT. In children with positive SPT to milk and/or egg, the food was eliminated from the diet. In food-allergic children, milk and egg were reintroduced in either open standardized or double-blind placebo-controlled oral food challenges when SPT was ≤10 mm and SCORAD ≤25 points.

At 4 ½ years of age we conducted a follow-up in all the children to assess eczema and carried out SPT tests for milk, egg and aeroallergens. We enquired about other allergic manifestations and clinical tolerance to foods. A pediatrician conducted the examination both at referral and at follow-up. Two children from the SPT-positive group and six from the SPT-negative group were lost to follow-up.

Statistics
For statistical analysis, the Chi2-test and Mann-Whitney U-test were used. Differences associated with p values of less than 0.05 (2-tailed) were considered significant.

Ethics

The Human Research Ethics Committees at the Faculty of Health Sciences, Linköping University, and at the Medical Faculty at Uppsala University approved the study.
Informed consent was obtained from the children's parents.

Results

At first visit

In 123 children referred because of eczema and suspected food allergy, 78 tested SPT positive to milk and/or egg, and 45 tested SPT negative. The respective ages of the two groups at referral were six months (median, range 1-23) and seven months (median, range 2-23).

The SPT positive children were prescribed a diet minus the corresponding food/s. Children in both groups were given a regime of skin lubrication and steroid topical treatment when required. The outcome after six weeks treatment of eczema is described previously (17).

The Hanifin-Rajka criteria for atopic eczema were fulfilled in 75/78 (96%) in the SPT- positive children and in 25/45 (56%) in the SPT-negative children ($p<.001$).

The groups differed significantly in severity of eczema scores, with more severe disease in the food-sensitized children, 19.9; 0-77 (mean; range), compared with SPT-negative children 13.5; 7.0-50.8 ($p<.001$). In 11 children the SCORAD was 0 points. These 11 children had all expressed eczema on referral but had successfully treated the eczema before the visit to our clinic. The presence and severity of eczema are presented in Table 1.

In SPT-positive children, 72/78 (93%) had a positive family history of atopy (first-degree relative), and 76/78 (97%) were breastfed. In the SPT-negative children, the results were 41/45 (91%) and 43/45 (96%), respectively (n.s). There was no difference between the groups with respect to keeping pets, 12/78 (15%) and 7/45 (16%), respectively. In 5/19 families with pets, the child suffered from airway symptoms. These 5 children were all SPT- positive. Tobacco smoking at home was reported by four families, all with SPT-positive children. Three of these children had airway symptoms as well as eczema. There was no significant difference between SPT-positive and SPT-negative children regarding presence of airway symptoms, 22/78 (28%) and 9/45 (20%).

In 31/78 children the foods were re-introduced early either by the parents at home, who did not wait for a controlled challenge or did not comply with the elimination diet, or by open challenge after a shorter elimination period. These children were regarded as food sensitized but not food allergic. The remaining 47 milk and/or food-allergic children, in whom early food challenge could not be performed due to severe eczema, SPT > 10 mm or recent allergic reactions on accidental exposure to the offending foods, were re-evaluated annually. Eight children were excluded from challenge because of SPT > 10 mm and/or severe generalized allergic reactions on accidental exposure to the foods within the previous six months. The remaining 39 children underwent oral food challenge when the SPT was $\leq$ 10 mm and SCORAD $\leq$ 25. The outcome of the challenges is described previously in detail (18).

At follow-up at 4½ years of age

Significantly more children were still affected by eczema in the initially SPT- positive group compared with the children who remained SPT-negative at the 4½ year control, ($p<.05$). With respect to the severity of the eczema, the groups did not differ significantly. The presence and severity of eczema are presented in Table 1.

In the initially SPT-positive group, 33/76 (43%) were still sensitized to milk and/or egg, expressing positive SPT. None of the SPT-negative group had become sensitized to food allergens ($p< .001$). All 39/39 children in the SPT-negative group were able to drink milk, and 38/39 (97%) could eat egg. One child avoided egg because it caused gastrointestinal symptoms. The corresponding figures for the SPT-positive group were 67/76 (88%) and 61/76 (80%), respectively, for milk and egg, and for the food-allergic children 38/47 (81%) and 32/47 (68%), respectively. Comparing non-sensitized children with sensitized and with food-allergic children the differences were significant, $p<.05$ and $p<.01$, respectively.

SPT positivity to airborne allergens was expressed in 44/76 (58%) in the SPT-positive group; the corresponding figure in the SPT-negative group was 10/39 (26 %), (p<.001). With respect to the presence of airway symptoms, there was no significant difference between the groups, 27/76 (36%) and 9/39 (23%). Nor was there any difference between the groups with respect to keeping pets, 13/76 (17%) and 6/39 (15%), respectively. In 9/19 families with pets, the children suffered from asthma symptoms; 7 children from the SPT-positive group and 2 from the SPT-negative group. The four children living in homes in which tobacco was smoked all had asthma at the follow-up, and three were sensitized to airborne allergens.

With respect to absence of atopic symptoms at follow-up, the difference between the initially SPT- positive group and the group who remained SPT-negative at the 4½ year follow-up was significant (p<.05).

The presence of eczema, gastrointestinal symptoms and airway symptoms in both groups at referral and at follow-up is summarized in Figure 1.

Discussion

In this prospective study we followed 123 children from referral because of eczema and suspected food allergy until 4½ years of age. Seventy-eight of the children were SPT-positive to foods and 45 were SPT-negative. Of the 78 milk and/or egg sensitized children 47 were assessed as food-allergic. These 47 children with food allergy constitute 38% of the 123 with eczema, which concurs well with previously presented figures (4-6). It has been described that food allergy contributes to the severity of eczema (1). We found a significant difference in severity of eczema at referral when comparing children sensitized to milk and/or egg with non-sensitized children ($p<.001$), whereas there was no difference in severity at the follow-up. Complete remission of AE by 3 years of age has previously been shown in 43% of children with early AE (19). This was demonstrated in a population-based study evaluating a birth cohort of 1,314 children. In the present study, in children referred to pediatric clinics, the remission was somewhat lower, 38% for the whole group and 33% in SPT-positive children. However, in SPT-negative children the complete remission was 49%. The findings concur with previous reports that the prognosis of AE is determined by the presence of sensitization (19).

Most children will outgrow their allergy to milk. Different results in tolerance achievement in IgE-mediated milk allergy have been demonstrated, including 76% by age 3 (20), 56% by age 4 (21), 78% by age 6 (21), 38% by age 7 (22), and 57% by age 8 (23). In the present study, 81% of early sensitized and previously milk-allergic children achieved tolerance and were drinking milk at 4½ years of age. This is more than the other studies have shown and might have been achieved because we performed challenges early in children expressing SPT wheal size up to and including 10 mm. Like some of the other studies, ours was based on children under the care of an allergy specialist, indicating a more severe form of food allergy. Nevertheless, the numbers achieving tolerance are higher than those in several previous studies. Development of tolerance to egg has previously been reported in 30-44% by school age (24, 25). In the present study, 68% were tolerant at 4½ years of age. These children, expressing SPT wheal size up to and including 10 mm, were challenged early, but with baked, not raw, egg, which may explain the greater number of children who were able to eat egg.

The concept of the atopic march suggests that approximately 80% of children with AE will eventually develop asthma and/or AR, with many outgrowing their AE with the onset of respiratory allergy (1, 11). Sensitization to aeroallergens has been demonstrated in 48% of milk-allergic children at age 3 (20). We found a significant difference between the children early sensitized to foods compared with SPT-negative children in sensitization to airborne allergens at the 4½ year follow-up, 58% and 26% respectively ($p<.001$), but found no difference in symptoms of asthma/AR. The higher rate of sensitization suggests, however, that on following up this group at school age, the number with allergic airway symptoms may increase.

Conclusions

Several previous studies have described the relations between early eczema, food sensitization and the atopic march (19-25). Compared with these studies, the main difference in our study was the high achievement of food tolerance at age 4½ years in children early sensitized and allergic to milk/egg. We presume that our active challenge procedure, despite remaining moderate positive SPT, contributed to the favorable prognosis. Further, we found a low rate of asthma/AR in children sensitized to aeroallergens. This might be explained by the relatively low age of the children at follow-up, as allergic airway disease often presents at a

later age. The low rate of exposure to tobacco smoke might be a further contributing factor (26).
Early identification of children with food allergy and AE are important as it might provide opportunities to prevent the development of asthma (2, 3, 12). These suggested interventions include eliminating exposure to tobacco smoke, specific allergen avoidance measures, dietary management, and pharmacotherapy involving intervention with H1-antihistamines in sensitized children. Recommending breastfeeding for the first 4-6 months of life, giving an extensively hydrolyzed formula to high-risk children if supplement is needed, and avoiding all exposure to tobacco smoke are well documented primary interventions (8, 26, 27, 28, 29). Further studies of the benefits and importance of other interventions are still warranted.

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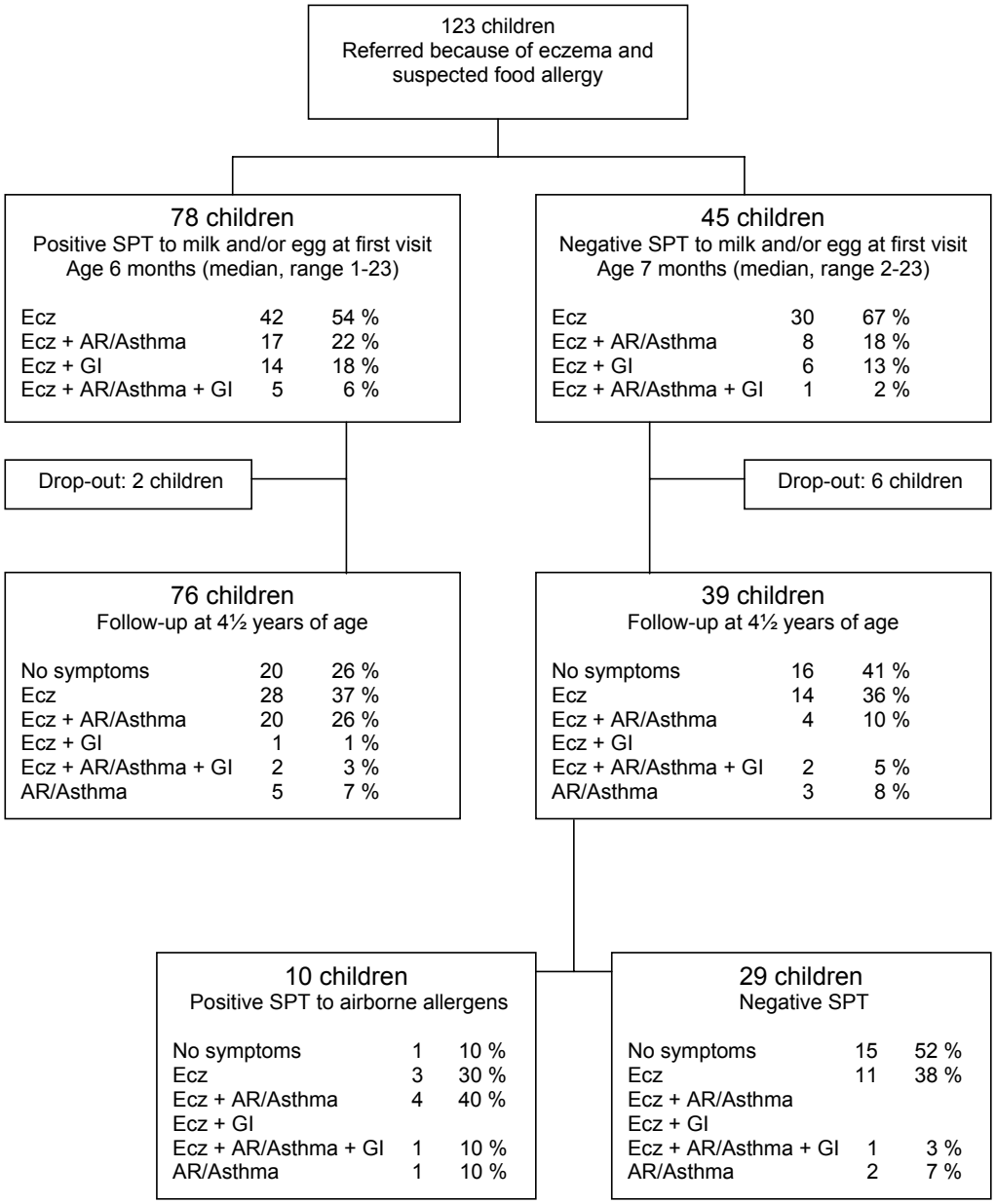
Fig.1

Presence of eczema (Ecz), gastrointestinal symptoms (GI) and asthma/allergic rhinitis (AR) at referral and at the 4½-year follow-up.



SPT = Skin prick test, Ecz = Eczema, AR = Allergic Rhinitis, GI = Gastrointestinal symptoms

Table 1

Severity Scoring of the Eczema (SCORAD)

	SPT positive when tested at first visit	SPT negative when tested at first visit
At first visit		
Mild eczema 1-25 points	42/78 (54%)	38/45 (85%)
Moderate eczema 26-50 points	28/78 (36%)	6/45 (13%)
Severe eczema > 50 points	8/78 (10%)	1/45 (2%)
At 4½ year follow-up		
Mild eczema 1-25 points	45/76 (59%)	19/39 (49%)
Moderate eczema 26-50 points	6/76 (8%)	1/39 (3%)
Severe eczema > 50 points	0	0

The value of SCORAD points in children, tested SPT positive and SPT negative at the first visit, were 19.9; 0-77 (median, range) and 13.5; 7.0-50.8, respectively ($p<.001$), on referral. The corresponding values for the same groups at the 4½ year follow-up were 0.05; 0-38.5 and 0.6; 0-28, n.s.

SPT = skin prick test, SCORAD = Severity Scoring of Atopic Dermatitis