Evaluation of severity and therapy in children with atopic dermatitis

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Cover illustration: Skin lesions of a child with atopic dermatitis

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Evaluatie van de ernst en therapie
van atopisch eczeem bij kinderen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit van Rotterdam
op gezag van de Rector Magnificus
Prof. dr. P.W.C. Akkermans M.A.
en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 8 september 1999 om 9.45 uur.

door
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geboren te Wenen

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Chapter 1

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Introduction

Atopic dermatitis (AD) is a common chronically relapsing skin disorder affecting 9–20% of those born after 1970 [Schultz Larsen 1993]. The aetiology is still not entirely elucidated and research is complicated by the multifactorial nature of the disease. Both genetical and environmental factors are involved in the pathogenesis of AD. The prevalence of atopic dermatitis seems to have increased along with asthma and allergic rhinitis during the past three decades [Williams 1992, Schultz Larsen 1996]. Several studies from different countries reported a two- to three-fold increase of the prevalence of AD over the past three decades. However, the reasons for this evolution of atopic diseases still remain to be elucidated. Furthermore, large, unexplained variations in prevalence have been reported between countries and within countries [ISAAC 1998], suggesting a critical role for environmental factors in disease expression. Although some risk factors such as gender, parental smoking, and early exposure to allergens (house dust mite, pets, cow's milk and solid food) have been identified, the role of other risk factors like socio-economic status, outdoor and indoor pollution and infections in early life are still a matter of discussion.

Studies on the genetical and immunological background have provided new insights into the mechanisms involved in atopic diseases. However, therapeutical practice has not yet changed. Recently guidelines based on consensus have been established for the management of AD [Mc Henry 1995]. Emphasis is put on educating and informing the patients. Although these and other guidelines provide a good framework for managing AD, the unpredictable course of the disease with exacerbations and remissions may frustrate both patients and physicians [Przybilla 1994].

Patients with AD account for about 30% of dermatological consultations in general practice, and dermatological consultations account for about 20% of all consultations in general practice [Rook 1986]. However, little attention has been paid to AD in terms of research. A Medline literature search (title, abstract, and subject heading) from 1996 to May 1999 showed 8,986 publications related to asthma, but only 942 related to AD. This is surprising when the impact of the two diseases is compared. In terms of prevalence, AD is more common than asthma in young children [Peat 1994, Burr 1989]; in terms of economic resources, the direct financial cost

in the care of a child with moderate to severe AD is substantially higher than for the average child with asthma [Su 1997]; and in terms of family impact - taking into account financial burden, familial/social impact, personal strain and mastery - even in mild AD, the impact on families was found to be equivalent to that for children with insulin dependent diabetes mellitus [Su 1997]. Consequently AD should not be perceived as a minor skin disorder, but it should be recognised as a disease with considerable social, personal and financial burden.

History of atopic dermatitis

> *His body is said to have been marred by.... a number of hard, dry patches suggesting ringworm, caused by an itching of his skin and a too vigorous use of the scraper at the baths...in early spring a tightness of the diaphragm; and when the scirocco blew, catarrh. (Suetonius A.D. 69-140 on the roman emperor Augustus Caesar) [Mier 1975]*

To our knowledge this is the first description of atopic dermatitis. Mier [1975] concludes that the combination of lichenified itching skin lesions, seasonal dyspnoea and rhinitis clearly indicates atopic diseases and must be the earliest recorded description of the atopic syndrome. Except for anecdotal evidence, we know nothing about the incidence of AD in the remote past. Ferdinand von Hebra gave the first detailed description of AD in 1884. He described an itchy papular eruption that began in early childhood, located in the flexures and persisted throughout life with interspersed exacerbations and remissions. Sulzberger and Wise first proposed the term atopic dermatitis in 1933 [Wise 1933]. A prerequisite was the work of Coca and Cooke who introduced the term atopy in 1923 [Coca 1923]. Atopy is derived from the Greek words a (no) topos (place). Initially atopy was meant to described the inherited tendency to develop immediate-type hypersensitivity reactions to common antigens. Atopy was found to be associated with an increased ability to form "reagins" (IgE antibodies). In the course of time many names have been given to the itching disease we now know as atopic dermatitis (Table 1) and other definitions of atopy followed. Another important term, allergy, was introduced by Clemens von Pirquet in 1906 as a deviation from the expected immunological reaction. Today, allergy is often used synonymously with hypersensitivity and atopy. Initially, von Pirquet meant to describe a condition of changed reactivity, irrespective of whether it was harmful (hypersensitivity) or protective (immunity).

In 1980, Hanifin and Raijka established the classical diagnostic criteria which enabled the comparison of studies on AD. In contrast, the terms allergy and atopy were defined and redefined several times, resulting in a Babylonian confusion of languages that still persists.

Table 1.   Nomenclature of atopic dermatitis

Synonym	Author
Mycoses flexurarum	Hans von Hebra
Prurigo	Ferdinand von Hebra
Prurigo diathésique	Ernest Besnier
Dermatitis lichenoides pruriens	Albert Neisser
Prurigo Besnier	Carl Rasch
Le prurigo de Besnier	H.R. Haxthausen
Konstitutionelles ekzem	A.G. Kochs
Neurodermatitis constitutionalis	U.W. Schnyder
Le prurigo-asthme	Raymond Sabouraud
Spätexsudatives Eczematoid	Geory A. Rost

Clinical manifestations

Atopic dermatitis (AD) starts in infancy in 60% of the patients and before the age of 5 years in 90% of the children [Su et al 1997]. The course of the disease is chronically relapsing with unpredictable exacerbations [Przybilla et al1994]. AD is characterised by a large number of clinical features. None of these features is pathognomic for the disease. Therefore, a combination of different symptoms is necessary for the diagnosis of AD. In 1980, Hanifin and Raijka established major and minor criteria for the diagnosis of AD (Table 2). These criteria are regarded as classical and have been used in nearly all the clinical trials on AD. However, sensitivity and specificity of the criteria and of their combination were not satisfactorily investigated. Some of the criteria turned out to have very low specificity. Therefore, the value of these criteria has been questioned and new, more practical ones have been developed. Sampson [Sampson 1990] compiled criteria for children younger than 2 years, whereas the criteria by Williams et al [Williams et al 1994] are used for individuals older than 2 years (Table 3 A&B). The criteria by Williams et al are similar in sensitivity to those by Hanifin and Raijka, but have a higher specificity. The diagnosis of AD is thus based on clinical criteria while the new knowledge on the pathophysiology has not been considered. Recently, there is discussion on including a parameter of atopy into the diagnostic criteria. Bos et al suggested that the presence of specific IgE should be a mandatory criterion for the diagnosis of AD (Table 4). Indeed about 80% of the patients with AD have increased levels of total or specific IgE. As a consequence, the remaining group of patients with normal levels of IgE needs to be addressed separately. Bos et al [1998] suggested to use the term atopic dermatitis when specific IgE is detectable and to use the term intrinsic atopic dermatitis or constitutional eczema for those with normal IgE. However, the classification of AD into atopic and non-atopic remains controversial, as there is no difference between those groups in terms of clinical symptoms or treatment.

The morphological lesions in AD cover a wide spectrum and change continually over time (Table 5). Usually, the lesions are symmetrical and not sharply demarcated. A selection of these cutaneous manifestations is used to score the intensity of AD.

Assessing the severity of AD should be considered as separate from establishing the diagnosis. Skin diseases offer the opportunity to score the severity of disease just by observation. However, standardisation and validation of the scoring system and training of the observer are necessary for this purpose. Such a scoring system for the severity of AD is crucial for investigating the efficacy of therapy. For clinical trials the SCORAD index has been established [European Task Force on AD 1993, Kunz et al 1997, Oranje et al 1997]. It is the only system based on an international consensus and validation. Like most of the other scoring systems, the SCORAD index is rather time-consuming and therefore not suited for daily practice. However, in a fluctuating disease such as AD, daily practice may be improved by using a more objective way of recording disease than by stating in the dossier "the eczema is better or worse". For daily routine the Three Item Severity (TIS) score has been developed [Wolkerstorfer et al 1999].

In conclusion, the skin lesions of AD allow both diagnosis and scoring of the severity of the disease which is important not only in basic research, but also in clinical practice.

The course of the disease is unpredictable with exacerbations and remissions. AD often starts during the first six months of life on the exposed areas of the body, whereas the diaper area is typically spared. The lesions become more distally located in the following years. Typical flexural involvement usually appears after the age of one year and lichenification may appear after the age of 2 years.

Complications and related diseases: Patients with AD have increased susceptibility for specific bacterial infections (Staphylococcus aureus) and specific viral infections (Herpes simplex). The course of these infections is usually more severe in individuals with AD, as compared to individuals without AD. Asthma develops in about 40% of the children with AD, whereas in about 30% allergic rhinitis develops [Bergmann 1998].

Prognosis: Spontaneous resolution may occur at any age, but cannot be predicted in individual patients. Reliable data on the evolution of AD from infancy into adulthood are hardly available. In a prospective study, resolution of AD was reported to occur in almost 90% of the patients during the subsequent 15 years [Vickers 1980]. However, most investigators reported a higher percentage of persistence (50 to 60%). In a retrospective study of individuals who were diagnosed as having AD at the age of 2 years, 72% still had signs of AD after 20 years

[Kissling et al]. Generally AD tends to improve with age. Patients with severe AD seem to have less chance to outgrow the disease [Guillet et al 1992].

Table 2.   Diagnostic criteria for atopic dermatitis by Hanifin and Raijka

Major criteria	Minor criteria
Pruritus	Xerosis
Typical morphology and distribution	Ichthyosis/keratosis pilaris/palmar hyperlinearity
Adults:Flexural lichenification and linearity	Type 1 skin test reactivity
Children: Facial, extensor	Elevated serum IgE
Chronic or relapsing dermatitis	Early age of onset
Personal or family history of atopy	Tendency to skin infection (Staph. aureus,
	Herpes simplex) / impaired cell-mediated
	immunity
	Tendency to hand/foot dermatitis
	Nipple eczema
	Cheilitis
	Recurrent conjunctivitis
	Dennie-Morgan fold
	Keratoconus
	Anterior subcapsular cataracts
	Orbital darkening
	Facial pallor/erythema
	Pityriasis alba
	Anterior neck folds
	Itch when sweating
	Intolerance to wool and lipid solvents
	Perifollicular accentuation
	Food intolerance
	Course influenced by environmental/emotional
	Factors
	White dermographism/delayed blanch

For the diagnosis of AD $\geq$ 3 major and $\geq$ 3 minor criteria are necessary

Table 3A.   Diagnostic criteria for atopic dermatitis
(>2 years) by Williams et al [1994]

Must have

❑   An itchy skin condition (or report of scratching or rubbing
in a child)

Plus three or more of the following

❑   History of itchiness in skin creases such as folds of the
elbows, behind the knees, fronts of ankles, or around neck
(or the cheeks in children under 4 years)
❑   History of asthma or hay fever (or history of atopic disease
in a first degree relative in children under 4 years)
❑   General dry skin in the past year
❑   Visible flexural eczema (or eczema affecting the cheeks or
forehead and outer limbs in children under 4 years)
❑   Onset in the first two years of life (not always diagnostic in
children under 4 years)

Table 3B.   Diagnostic criteria for atopic dermatitis
(<2 years) by Sampson [1990]

Major features

❑   Family history of atopic dermatitis
❑   Evidence of pruritic dermatitis
❑   Typical facial or extensor eczematous or lichenified
dermatitis

Minor features

❑   Xerosis/ichthyosis/hyperlinear palms
❑   Peri-auricular fissures
❑   Chronic scalp scaling .
❑   Perifollicular accentuation

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Table 4.   Millennium criteria for the diagnosis of atopic dermatitis [Bos 1998]

Mandatory criterion	Principal criteria (2 of 3 present)
❑ Presence of allergen-specific IgE ❑ Historical, actual, or expected (in very young children) ❑ In peripheral blood (RAST, ELISA) or in skin (intracutaneous challenge)	❑ Typical distribution and morphology of eczema lesions: infant, childhood, or adult type ❑ If distribution is not typical, exclude other entity (dyshidrotic eczema, contact dermatitis, contact urticaria ❑ Pruritus ❑ Chronic or chronically relapsing course

Table 5.   Morphological elements in atopic dermatitis

Acute ↔	Subacute ↔	Chronic
❑ Erythema	❑ Crusts	❑ Dryness / Scaling
❑ Oozing	❑ Oedema	❑ Hyper-hypopigmentation
❑ Vesicles	❑ Papulation	❑ Lichenification
❑ Excoriations	❑ Fissures	

Epidemiology

Atopic dermatitis is a common skin disease affecting 9–20% of those born after 1970 [Schultz Larsen 1993]. The prevalence in Northern Europe was found to be 16% among 7-year-old children born in 1985 in Germany, Sweden and Denmark [Schultz Larsen 1996]. This is confirmed by another study reporting a prevalence of 20% for children born in the 1980s in the U.K.

A systematic, world-wide comparison of the prevalence of atopic diseases has recently become available [Lancet 1998, Williams et al 1999]]. The International Study of Asthma and Allergies in Childhood (ISAAC) provided a core questionnaire to assess the prevalence of asthma, allergic rhinitits and AD (Table 6) [ERS 1995]. Although there may be some objections on diagnosing AD via questionnaire, it does provide large scale data on the prevalence of AD.

Table 6.   ISAAC questionnaire for the diagnosis of AD in children

The following questions were asked to the parents of 6 to 7-year-old children and to 13 to 14-year-old children:

1. Have you (Has your child) ever had an itchy rash that was coming and going for at least 6 months?
 2. Have you (Has your child) had this itchy rash at any time in the last 12 months?
 3. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or the eyes?
 If yes to question 3:
      In the last 12 months, how often, on average, have you (Has your child) been kept awake at night by this itchy rash? (- never in the last 12 months, - less than one night per week, - 1 or more nights per week)
 All respondents were asked:
      Have you (Has your child) ever had eczema?

Recently, the analysis of the immense database concerning 715,033 children in 56 countries has been published. As expected, this global survey revealed higher prevalences in Northern Europe, Australia, New Zealand and Japan, and lower prevalences in Eastern Europe, China and Central Asia. Surprisingly, data from some African countries suggested high prevalences. Furthermore variations within countries were found. Interestingly, countries with the highest prevalence rates were shown to have the highest IgE levels in a recent standardised international comparison [Burney 1997].

However, it must be added that a questionnaire validated in one country may have different sensitivity and specificity in another country. Such as arthropod infections that rarely occur in developed countries may be confused with AD in developing countries.

There is general agreement that the prevalence of atopic dermatitis has increased dramatically during the past decades [Schutz Larsen 1996] (Table 7). This trend goes along with an increase in other atopic disorders, asthma and allergic rhinitis [Sears et al 1997, Burr et al 1989, Ninan et al 1992] (Fig 1).

However, comparative epidemiological studies over time may be biased due to the following reasons (Williams 1992):

- different definitions of the disease
- changes in diagnostic procedures
- changes in the acceptability of eczema as a diagnosis (in earlier years eczema used to be associated with uncleanliness and arthropod infestation)
- increased awareness of both patients and physicians
- recall bias

Further evidence for an increase of the prevalence of atopic diseases comes from studies that investigated the change in the expression of biological markers using the same methodology over time [Burr et al 1989, Peat et al 1994, Sibbald et al 1990, Barbee et al 1987, Nakagomi et al 1994].

In two surveys 10 years apart, Peat et al [1994], reported in children an increase in airway hyperresponsiveness of 2-fold in Belmont and 1.4-fold in Wagga Wagga. Burr et al [1998], reported that the number of children with an impaired exercise provocation test was higher in 1988 than in 1973. They concluded that if the response to exercise indicates severity of

asthma, both mild and severe asthma had become more common. Sibbald et al [1990], investigated skin prick test reactions using the same methodology and allergens in 1974 and 1988. They reported an increase in the proportion of subjects with at least one positive skin prick test reaction from 23% in 1974 to 46% in 1988. Similar results were published for an increase of specific IgE levels to common allergens [Nakagomi et al 1994].

The conclusion of these studies was that the increase in prevalence may partly be due to methodological bias, but also reflected a true increase of atopic diseases and sensitisation.

Table 7.   Evidence for increasing prevalence of AD

	Country and Time span	Age	Prevalence
Peat et al, 1994	Australia 1982→1992	8-10 years From 2 towns	22.5%→31.9% (Wagga Wagga) 20.3%→24.4% (Belmont)
Taylor et al, 1984	U.K. 1946→ 1958→1970	5-7 years	5.1%→ 7.3%→12.2%
Burr et al, 1989	U.K. 1973→1988	12 years	4.8%→15.9%
Schultz Larsen et al, 1986	1964-69→ 1970-74	7 years	3%→10%
Ninan et al, 1992	U.K. 1964→1989	8-13 years	5.3%→12%

The reasons for the increased prevalence of atopic diseases are still a matter of discussion. According to the current understanding the genetic background has not changed. Thus environmental factors must be responsible.

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Maternal smoking during pregnancy has presumably increased. It was documented that smoking among women of childbearing age has increased considerably during the past three decades and there is evidence to show an increased risk for asthma and AD when the mother smokes during pregnancy [Ronmark et al 1998, Gergen et al 1998, Hu et al 1997].

Another theory concentrates on a change in *indoor environment*. Some of the major allergens like house dust mite and pets are found in the houses. Recently, other relevant indoor allergens (cockroaches and fungi) have been identified. Because young infants spend most of the time indoors, the exposure to these allergens is high. A change in housing conditions such as central heating, better isolation, less ventilation, and wall-to-wall carpeting resulted in higher concentrations of house dust mite. Peat et al [1994] found that the increase of AD, wheeze and airway hyperresponsiveness was paralleled by an increase in house dust mite concentrations. In the course of 10 years the concentrations of house dust mite increased 5.5-fold in Belmont and 4.5-fold in Wagga Wagga [Peat et al 1994]. Similarly, in New Guinea the increase of asthma was paralleled by an increase in the concetration of house dust mite (Dowse 1985). On the one hand, these studies point to a pathogenic role for house dust mite allergens in the increase of asthma. On the other hand, high increases in the prevalence of AD and asthma have been observed in regions where the levels of house dust mite are still low (e.g. in the northern parts of Sweden). Furthermore, it was observed that in regions with very low humidity and low house dust mite concentrations other allergens dominated. Thus, it may be that an allergy-prone immune system will anyway get sensitised to any available allergen.

Although there is no definite proof, some authors suggested that a change of nutrition in early life may partly be responsible for the increasing prevalence of AD. Foods have been implicated in the development of atopic disease through the following mechanisms. 1) Early introduction of food allergens may be the first trigger in the development of atopy. 2) A specific deficiency of polyunsaturated essential fatty acids (PUFAs) has been found in the blood of patients with AD. Moreover, the composition of the breast milk with regard to essential fatty acids differed between atopic and non-atopic infants. PUFAs may be involved in the regulation of the Th1/Th2 balance. It was suggested that an increase in the consumption of omega-6 PUFAs like linoleic acid (found in margarine and vegetable oils) and a decrease in consumption of omega-3 PUFAs like eicosapentaenoic acid (found in fish)

is the reason for the increased prevalence, social class differences, and regional differences of atopic diseases [Black et al 1997]. The pathogenic background of this hypothesis is that linoleic acid is a precursor of prostaglandin E2 (PGE2) which inhibits the formation of interferon gamma (IFN-γ), while eicosapentaenoic acid inhibits the formation of PGE2 [Black 1999]. 3) Food additives in processed foods may be one of the unidentified risk factors linked to the modern Western lifestyle.

Outdoor pollution was thought to play a dominant role. Different experimental studies demonstrated that pollutants have a promoting effect on sensitisation. However, the East/West German comparison studies by von Mutius et al [1992, 1994, 1998] have changed our view. Surprisingly they noted lower prevalences of asthma, hay fever, airway-hyperresponsiveness, and atopic sensitisation (prick test) in the polluted cities of East Germany (Leipzig, Halle) as compared with a less polluted West German city (Munich) (von Mutius et al 1994, von Mutius et al 1992). The strikingly high frequency of sensitisation to house dust mite, cat and pollen in West Germany could explain a large part of the difference in asthma and hay fever between East and West Germany (von Mutius et al 1994). In a logistic regression model, West German origin was no longer an independent risk factor when sensitisation was taken into account (von Mutius et al 1994). They hypothesised that "Western lifestyle" was a major risk factor for atopic diseases, whereas outdoor pollution was not. Interestingly, in another study, they observed an increase in the prevalence of hay fever and atopic sensitisation in East Germany since the reunification. This may be explained by the exposure to risk factors associated with the "Western lifestyle" (von Mutius et al 1998).

Still, certain phenomena like the dramatic increase of cedar pollinosis in Japan cannot be explained by any of the above mentioned factors. While the exposure to pollen of the Japanese cedar tree has remained stable, the sensitisation has increased from 8.7% to 36.7% in 1985. The only conclusion from this observation - except methodological bias - is that the population has become more susceptible. A hypothesis that may explain this increase in susceptibility is based on the balance between Th1 and Th2 type cytokines.

The reduced number of *infections in early childhood* may have skewed the balance towards Th2 type cytokines resulting in an allergy-prone immune system. This hypothesis is supported by epidemiological studies that demonstrated that the number of older siblings was

negatively correlated to the risk for atopic diseases. Further support comes from the observation that certain infections (measles, tuberculosis) seemed to decrease the risk for atopic diseases (Shaheen et al 1997, Shirakawa et al 1997). Established atopic disease was demonstrated to improve by helminth infections (Turton et al 1976). The increased use of antibiotics, vaccinations and the higher socio-economic standard has led to a decline in infections which may favour a Th2 type cytokine pattern associated with atopic diseases. Despite the existing evidence in favour of this hypothsis, proof has still to be furnished.

In conclusion, AD is a world-wide problem that is still increasing without any evident satisfactory reason reasons for this increase.

Risk factors - Prevention

The increase in atopic diseases during the past three decades underlines the importance of environmental factors. Some of these risk factors may be subject of preventive measures. In course of time there have been many claims, but few proofs of risk factors for the development of AD. Caution with the interpretation is necessary as epidemiological studies can only detect associations, and not causal relations. Associations found in one population may not be present in other populations. Only a few risk factors like smoking have been found consistently in different populations, which implies that they must be genuine.

1. Risk factors with potential for primary prevention

1.1 Allergens: Early exposure to allergens in predisposed individuals is generally perceived as a major contributor in the development of atopic diseases [Hide et al 1996]. Consequently, most of the preventive efforts are directed at the reduction of exposure to allergens in early life.

- Food allergens: Food allergy occurs in about 8% of the general population of children and in about 20% of the children with AD [Pearl 1997, Bock 1987]. Food allergy seems to be more prevalent in children with severe AD [Sampson 1992, Guillet et al 1992] than in mild AD. Eigenmann et al [1998] reported that 37% of the children with moderate severe AD had clinically significant food allergy. The early introduction of cow's milk and solid food has been implicated in the development of food allergy and subsequent atopic disease. Food allergens may be one of the initial triggering factors for AD in infancy [Sigurs et al 1994]. This is supported by studies in both unselected cohorts and in high-risk children that demonstrate the predictive value of sensitisation to food allergens for the development of atopic diseases (Zeiger et al 1995, Hattevig et al 1987, Kulig et al 1998). Zeiger et al found that food allergy by 4 years almost doubled the period prevalence between 4 and 7 years of allergic rhinitis and asthma. Food allergy is often regarded as the first manifestation of the atopic syndrome [Kjellman 1998]. Therefore, many studies aimed at reducing the prevalence of atopic diseases through dietary measures in early life:

Different preventive measures have been investigated:

1) Diet during the last trimester of pregnancy

2) Diet during lactation

3) Encouraging breast-feeding or a hypoallergenic formula if breast-feeding is not possible or both

4) Delayed introduction of solid food until 5-6 months of age

5) Avoidance of potent dietary antigens during the first year of life

Although prenatal sensitisation to foods may occur [Jacobsen et al 1995], a diet during pregnancy, can not be advised based on efficacy and possible nutritional hazards to the mother [Falth-Magnusson et al 1992, Lilja et al 1988]. A diet during lactation may have some effect on AD, but must still be regarded an experimental approach (Zeiger et al 1995). The possible hazards of dietary exclusion to mothers and infants must be prevented by appropriate medical and dietetic support. The other three approaches, 3 to 5, are often combined and are, despite a large number of clinical trials, still controversial.

One of the earliest studies in this field dates back to 1936 and investigated the protective influence of breast-feeding [Gruele et al 1936]. In a study of 20,061 infants, a marked difference in the prevalence of eczema was found between exclusively breast-fed infants, partially breast-fed infants (twice the prevalence compared to exclusively breast-fed children) and never breast-fed children (seven times the prevalence). Since then, such a such a pronounced protective effect has not been confirmed [Golding 1997]. Fergusson et al [1990] studied 1265 infants in a prospective study and demonstrated that the number of different foods introduced in the first 4 months of life predicted the development of AD. Saarinen et al [1995] reported a long-lasting preventive effect of breast-feeding on the manifestation of atopic diseases (AD, asthma, allergic rhinitis) up to the age of 17 years. However, their study, such as many others may have been biased by the absence of a randomisation. This is relevant, because mothers who tend to give breastfeeding may differ in many confounding factors from mothers who do not.

Zeiger et al [1995] claim to have conducted the study with the largest cohort of high risk infants in a randomised, controlled setting to evaluate the effect of combined maternal and infant food allergen avoidance. In the prophylactic group, mothers avoided cow's milk, egg and peanut during the last trimester of pregnancy and lactation, and infants

avoided cow's milk (<1 year), egg (<2 years) and peanut and fish (< 3 years). Results were evaluated after a follow-up of 1-year [Zeiger et al 1989], 4 years [Zeiger et al 1992] and 7 years [Zeiger et al 1995]. They observed a temporary reduction in food allergy and sensitisation to cow's milk at the age of 1 year, with a diminution of the benefit by 2 years and an absence of any benefit at the ages of 4 and 7 years. In a recent review of the literature, Golding et al [1997] found little consistent evidence to support a persisting protective effect of breast-feeding on atopic diseases.

Many investigators now think that dietary prevention is temporarily effective with regard to food allergy and atopic dermatitis during the first years of life, while there seems to be no effect on the prevention of respiratory allergy [Wood et al 1996, Hide et al 1996]. This is in line with the phenomenon that most of the children outgrow food allergy to cow's milk and egg by the age of 2 to 4 years.

However, breast-feeding has such major benefits by preventing infections and promoting the mother-child-tie that no further justification is needed to advise breast-feeding.

- House dust mite: While food allergens play a role in young infants with AD, house dust mite allergens may be implicated in older children and adults with AD [Kapp 1995]. Atopy patch tests with house dust mite give rise to eczematous lesions in some of the patients with AD. It has been documented that house dust mite is an important trigger for AD [Cameron 1997], but we do not know whether the exposure to house dust mite promotes the development of AD. A double-blind controlled study demonstrated the benefit of avoidance measures (mattress covers) on adults with AD [Tan et al 1996]. Sporik et al [1990] have shown that the house dust mite concentration at the age of one predicts the development of wheezing at the age of 11 years. We have no information whether this is true for AD as well. In the ETAC study, it was shown that carpets in bedrooms were associated with a higher risk for sensitisation to house dust mite. Furthermore, the ETAC study demonstrated an increased risk for asthma in children sensitised to house dust mite. Indirect evidence in favour of a preventive effect comes from combined avoidance protocols for both food and inhalant allergens, which were associated with a long-lasting reduction of both sensitisation to foods and prevalence of AD [Hide et al 1996, Marini 1996]. Furthermore it was shown that the severity of AD increases with the sensitisation to house dust mite [Guillet ae al 1992]. However, to date

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there is no direct proof that reducing the house dust mite exposure prevents any atopic disease.

- Pets: The increased risk for AD in the families with pets has been documented in several studies. Kalyoncu et al [1994] found that the cumulative prevalences of childhood AD was significantly associated with the presence of pets at home. The ETAC study demonstrated that a sensitisation to cat allergens at the age of 1 to 2 years predicted the development of asthma in children with AD and a family history of atopy during the subsequent 18 months [ETAC 1998].

1.2 Indoor environment

- Passive smoking: There is overwhelming evidence that passive smoking increases the risk for all atopic diseases [Kalyoncu et al 1994, Schafer et al 1997, Gergen et al 1998, Ronmark et al 1998, Hu et al 1997]. The relationship is consistent in different studies. Smoking during pregnancy, lactation and in infancy has been demonstrated to be independent risk factors for the development of AD. Schafer et al [1997] reported that the risk for AD in pre-school-age children doubled when the mother smoked during pregnancy and/or lactation. Consequently, this should be a focus of preventive measures.
- Gas cooking: Indoor use of gas without hood has been associated with a higher prevalence of AD [Schafer et al 1996]. This is in line with asthma where exposure to gas stoves was shown to increase the risk for respiratory symptoms [Garrett et al 1998,] Jarvis et al 1996].

1.3 Outdoor environment

Although air pollution seems not to have a major role in the development of AD, it may aggravate the condition by acting as non-specific irritant and immunomodulator [Schafer et al 1997]. From animal and human studies it is known that sensitisation to allergens is enhanced by several pollutants, in particular, diesel particles (Suzuki et al 1989), SO_2 (Riedel et al 1988), NO2, and Ozone (Biagini et al 1986). These studies suggest that air pollution may promote the development of atopic diseases. However, in a series of studies comparing the prevalence of atopic diseases between East and West Germany, von Mutius et al [1992, 1994, 1998] reported a higher prevalence of sensitisation,

airway-hyperresponsiveness, asthma, and hay fever in the less polluted cities of West Germany. Regarding the prevalence of AD, these studies failed to show a significant difference between East and West Germany [von Mutius et al 1992, von Mutius et al 1998].

Schafer et al [1996] specifically addressed the prevalence of AD and compared various direct and indirect parameters of air pollution in an East-West German study. The highest association with AD was found in the use of gas stoves without hood and close proximity to roads with heavy traffic. Although sensitisation was more prevalent in West Germany, AD was more common in East Germany. This is in contrast to the studies reported by von Mutius et al [1992, 1994]. Schafer et al [1996] concluded that AD followed a course different from that of respiratory allergy and sensitisation. Furthermore, similar to that in other epidemiological studies, they failed to show a direct relationship between any measured pollutant (SO_2, suspended particles, NOx, dust fall) and AD. Thus, outdoor pollution as a risk factor is still a controversial issue. While experimental studies in animals and humans point to a pathogenic role for pollution in the development of atopic diseases, epidemiological studies fail to prove that association.

2. Other risk factors

- Positive family history

  Atopy often runs in families suggesting the importance of genetic factors. A positive family history for atopy seems to be the single most important predictor of AD [Arshad et al 1992, Selcuk et al 1997, Schafer et al 1997, Berth-Jones et al 1997, Tariq et al 1998].

  Dold et al [1992] calculated the relative risk for having a child with AD, allergic rhinitis or asthma:

	Relative risk (odds ratio=OR) for having a child with		
	AD	Asthma	Allergic rhinitis
One parent with AD	3.4	1	1.7
One parent with allergic rhinitis	1.4	1	3.6
One parent with asthma	1.5	2.6	2.5

In a comparable study, Uehara et al [1993] reported the prevalence for having a child with AD:

- Both parents with AD   81% prevalence
- One parent with AD   56% prevalence
- One parent with AD + one with respiratory atopy   59% prevalence

From these studies [Dold et al 1992, Uehara et al 1993] it may be concluded that the risk for AD increases with the number of affected first degree family members and that an organ-specific transmission occurs. This means that the risk for AD is particularly high if one or both of the parents have AD. Furthermore a maternal history of atopy has a higher predictive value than a paternal history [Dotterud et al 1995, Ruiz et al 1992]. This seems to be explained by influences in utero that may be related to a skewing in the T helper cell population of the foetoplacental unit.

- Socio-economic standard

AD has been termed a disease of the advantaged. Indeed, AD is more common in developed countries and to date the prevalence seems to be rising also in the developing countries. Furthermore, various studies from developed countries show an increased prevalence in higher socio-economic classes [Golding et al 1987]. Strachan et al [1997] reported an increased prevalence of positive skin tests in those with a higher socio-economic status in childhood. In a study of 8,279 British schoolchildren the prevalence of AD increased along with socio-economic class [Williams et al 1994]. The highest social class had almost twice the prevalence compared with the two lowest social classes. Importantly, the diagnosis of AD was not only based on self-reporting but also on medical examination, which excludes parental overreporting and better access to health services in higher classes as confounding factor. The reasons for this social class trend are unknown. Possible explanations are differences in housing conditions (presence of carpets, central heating), use of showers or soaps, contacts with pets, higher maternal age as well as differences in nutrition.

- Family size and birth order

A higher prevalence of atopic diseases has been described in first-borns and children from small families. Olesen et al]1997] calculated that the adjusted relative risk for a

specialist diagnosis of AD was 0.86, 0.69 and 0.26 when the mother had 2, 3 or 4 children, respectively. In another study, it was demonstrated that the prevalence of positive skin test results was independently correlated to a lower number of older siblings, but not younger siblings [Strachan et al 1997]. Bodner et al] 1998] reported that the risk for AD was inversely correlated to having had three or more older siblings (OR=0.3). Assuming that infections in early life are more frequent in children with older siblings, these studies support the impact of infections in early life in protecting against subsequent atopic diseases. The underlying hypothesis is that infections promote a Th1 type cytokine pattern that inhibits or prevents allergic reactions.

- Attendance to day care nursery

Recently, it was suggested that the attending day care nurseries at an early age may be associated with a protective effect on the development of allergy in later childhood [Kramer et al 1999]. This finding may also be explained by a protective effect of early infections. Kramer et al [1999] reported that the prevalence of a positive skin prick test was significantly lower in children who attended the day nurseries at an earlier age. Children who attend day care nurseries are known to have a higher risk for infections than those who do not. Kramer et al [1999] noted a reduction in the prevalence of atopy only in small families (up to 3 people), but not in large families. This may be explained because the effect of day nurseries on the frequency of respiratory illness is most pronounced if a child has no older siblings [Hurwitz et al 1991]

- Migration

Studies in migrant groups have shown large increases in the prevalence of AD compared with migrants country of origin, indicating the importance of socio-economic and environmental changes such as those associated with industrialisation [Mc Nally et al 1998]. Williams et al [1995] found that the prevalence of AD, diagnosed by a dermatologist on clinical aspects and criteria, was 16.3% in black Caribbean children and 8.7% in white children.

- Gender

  The female to male ratio of AD has recently been reported to be 1.3:1 in a global survey of 715,033 children. [Williams 1999]. The same preponderance of female gender was found in a survey in Northern Europe. However, some studies found a higher risk for AD in males (Raijka 1975). In contrast, asthma is consistently more common in boys, which is assumed to be due to relatively smaller airways in boys than in girls.

- High birth weight

  Olesen et al [1997] observed that a birth weight of $\geq$ 500g above expected could predict AD (relative risk = 1.59). However, this finding was not confirmed in other studies..

- Gestational age

  A Danish study in 7,862 children showed a statistically significant relationship between gestational age and a diagnosis of AD by a dermatologist (adjusted relative risk for gestation age $\geq$ 41 weeks = 1.32) [Olesen et al 1997].

- High maternal age

  Olesen et al [1996] observed an increased prevalence of AD when the mothers were older and suggested a dysmaturation of the immune system in children of older mothers. This risk factor could not be confirmed in a second study from the same investigators [Olesen et al 1997].

The increase of AD in the national British birth cohort (1974-1986) seems to have occurred in the context of a general increase in atopic disease and was largely unexplained by changes in the distribution of maternal age, birth order, birth weight, infant feeding, maternal smoking, active smoking by the child, or father's social class [Lewis et al 1996]. In that study the known risk factors did not explain the increase of atopic diseases noticed during the past decades. The challenge for future epidemiological research will be to combine epidemiological data from standardised questionnaires with immunological data concerning the underlying mechanisms.

Pathophysiology

Atopic diseases have a complex pathophysiology with a variety of both genetic and environmental factors being involved. Research on the pathophysiology of atopic dermatitis (AD) is complicated due to the many pathogenetic factors involved, the large number of described immunological changes and the various manifestations of the atopic syndrome.

A major concept on the pathophysiology of atopic diseases has evolved from the discovery that allergen-specific T cells generally belong to the T-helper type 2 (Th2) subset of lymphocytes, producing relatively high levels of IL-4, IL-5 and IL-13, but relatively low levels of interferon-gamma (IFN-γ) after stimulation [Wierenga et al 1990, Romagnani 1994]. Although there has been objections on the strict classification into discrete subsets, this model has profoundly stimulated the evolution of important principles in immune regulation [Kelso 1995]. One of the results of this concept was the unravelling of the mechanism and regulation of IgE synthesis.

Another important discovery relates to the function of adhesion molecules on leucocytes and endothelial cells in allergic inflammation. Skin lesions from patients with AD are characterised by an infiltrate of T cells, eosinophils and mast cells. The upregulation of endothelial adhesion molecules is crucial for the selective migration of leucocytes from the blood into peripheral tissues. In addition to adhesion molecules, chemokines promote the accumulation of leucocytes in inflammatory tissues. Chemokines mediate the extravascular migration of leucocytes (Rafeul 1997).

An important component in the pathophysiology of atopic diseases is exposure to allergens in predisposed individuals. It is documented that AD may deteriorate or even exacerbate after contact with an allergen. This phenomenon is the basis of the atopy patch test, which can be used as an experimental model for AD [Langeveld-Wildschut et al 1999]. Furthermore, it is documented that exposure to allergens increases the risk of sensitisation, and sensitisation increases the risk of atopic diseases.

The role of micro-organisms in the development of sensitisation and the pathophysiology of atopic diseases has been the focus of recent research. In particular, the colonisation of the skin with Staphylococcus aureus has been suggested to be responsible for a continuous
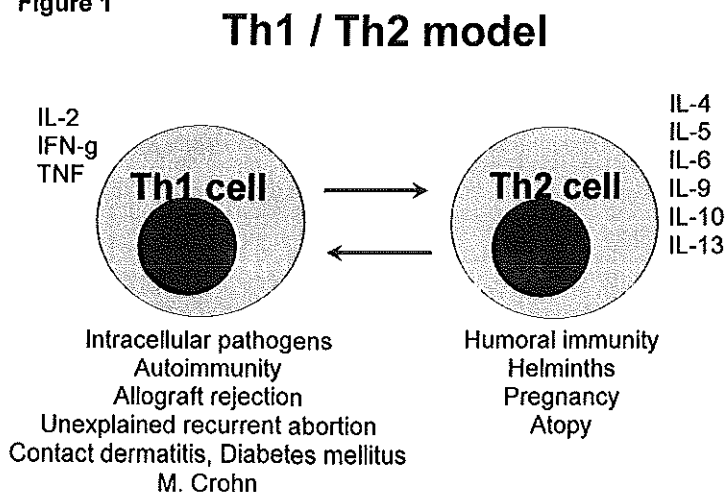
stimulation of T cells. Recently, research has focused on the genetic background of asthma and atopy, whereas little is known in AD.

- Th1 / Th2 - a working hypothesis

The concept of T helper cell subclasses was first proposed by Mosmann and colleagues in 1986. They reported that cloned CD4$^+$ T cell lines from mice could be classified into two subclasses according to their cytokine production and their related function [Mosmann et al 1986]

The key cytokines of Th1 and Th2 lymphocytes are IFN-γ and IL-4, respectively. These two cytokines were demonstrated to have opposite effects on a variety of immune mechanisms including IgE isotype switching in B cells, functional status of macrophages, and the development of T cell cytokines [Kelso 1995]. Figure 1 shows the cytokine pattern produced by Th1 and Th2 cells and the diseases in which a differential expression of either a Th type 1 or Th type 2 cytokine profile has been implicated.

Figure 1

Th1 / Th2 model

IL-2
IFN-g
TNF

Th1 cell → **Th2 cell**

IL-4
IL-5
IL-6
IL-9
IL-10
IL-13

Intracellular pathogens
Autoimmunity
Allograft rejection
Unexplained recurrent abortion
Contact dermatitis, Diabetes mellitus
M. Crohn

Humoral immunity
Helminths
Pregnancy
Atopy

In the absence of clear polarising signals, T cells with a less differentiated cytokine profile than Th1 or Th2 cells arise [Romagnani 1996]. These cells are called Th0 cells and can

develop further into Th cells producing a more polarised cytokine profile. However, it still is not clear whether the switch between Th1 and Th2 is a switch in individual cells or a population phenomenon, with more or less cells developing along the Th1 or Th2 pathway [Gallagher 1997].

However, the concept of a Th1/Th2 dichotomy turned out to be a simplification of the true nature of immune regulation. [Kelso 1995, Borish et al 1997, Allen et al 1997]. A rigid interpretation of the Th1/Th2 model has been criticised because the Th1/Th2 model was based on T cell clones cultured in vitro from mice. However, cloning is an artificial process and a polarised Th1 or Th2 pattern is found after long-lasting culture with certain cytokines or in T cell clones from some hyperimmune individuals. In contrast, T cell clones from normal individuals assayed early after culture coexpress Th1 and Th2 type cytokines in various combinations. Furthermore the situation in human beings is different from the murine situation as there is no clear-cut distinction between the cytokine profile of Th1 and Th2 cells. In human beings both "subsets" produce IL-2, IL-3, IL-10, IL-13, and GM-CSF.

Another limitation of the model is the diversity of the cytokine pattern produced by Th cells. It often is assumed that IL-4 represents a Th2 type cytokine profile, whereas IFN-γ represents a Th1 type cytokine profile. However, the expression of each cytokine seems to be regulated independently and Th cells have been described that contain mRNA for only one cytokine [Assenmacher et al 1994].

Therefore, it was proposed that Th1 and Th2 cells are not discrete subsets, but extreme phenotypes of a continuous spectrum in-between [Kelso 1995]. Interestingly, type 1 and type 2 cytokine patterns were not only found in CD4+ T cells, but also in the other T cell subsets such as CD8+ T cells and $\gamma\delta$ T cells (Ferrick et al 1995).

Campbell et al [1998] compared IL-4 and IFN-γ production from children with AD, children with IgE-mediated food hypersensitivity without AD, and normal controls. As compared with healthy children, children with AD had increased IL-4 production and decreased IFN-γ production, whereas children with IgE-mediated food hypersensitivity had increased IL-4 but normal IFN-γ (Campbell et al 1998). They suggested that isolated IL-4 enhancement promotes the development of IgE-mediated hypersensitivity, whereas the combination of

defective INF-γ and enhanced IL-4 production promotes inflammatory atopic disorders such as AD and asthma.

However, in AD Th1 type cytokines are important as well [Grewe et al 1994, Werfel et al 1996, Grewe et al 1998], and a two-phase-model has been proposed, with Th2-type cytokines promoting the initiation of the allergic inflammation and Th1-type cytokines later on prolonging and aggravating the allergic inflammation [Thepen 1996, Grewe 1995].

Several investigators reported that house dust mite-specificT cell clones from peripheral blood of patients with AD produced both IL-4 and IFN-γ [Werfel et al 1996]. In lesional skin in AD, Grewe et al [1994] reported increased levels of IFN-γ mRNA expression in 13 out of 15 patients, whereas increased levels of IL-4 mRNA expression were found in only 4 out of 15 patients as compared with normal skin. Furthermore, they observed that successful treatment reduced IFN-γ, but not IL-4 mRNA expression, indicating that in-situ IFN-γ expression is linked to the clinical course of AD. Thepen et al [1996] found a differential cytokine expression in APT tests depending on the timing of measurement. After 24 hours, they observed a predominance of IL-4 positive cells, whereas after 48 and 72 hours a predominance of IFN-γ positive cells was present. Moreover, they observed a predominance of IFN-γ positive cells over IL-4 positive cells in lesional skin of patients with AD [Thepen 1996]. Hamid et al [1994] compared the mRNA expression for IL-4, IFN-γ, and IL-5 in acute versus chronic lesions of AD. In acute lesions they observed a significantly higher number of cells positive for IL-4 and IL-5 mRNA, whereas in chronic lesions only IL-5 mRNA was increased [Hamid et al 1994].

Taken together, these studies support the concept of a sequential Th cell activation, with a Th2 type pattern in the initial phase and a Th1 type pattern in the chronic phase of AD [Grewe et al 1998, Thepen et al 1996]. In line with this concept is the observation in a mouse model, that injection of Th2 cells induced a rapid and short inflammatory response, whereas injection of Th1 cells into the skin induced a late onset and prolonged inflammatory response [Muller et al 1993]. The two-phase model is also reflected in a distinct expression of cytokine receptors. Taha et al [1998] reported that acute AD was associated with a high expression of IL-4 receptor alpha, whereas IL-5 receptor alpha and GM-CSF receptor alpha mRNA were predominantly increased in chronic AD and to lesser extent in acute lesions.

These findings support the biphasic role of IL-4, IL-5, and GM-CSF in the pathophysiology of AD.

The underlying mechanisms involved in the regulation of the sequential Th cell activation are not known. Recently it was speculated that IL-12 secreted by eosiniphils may be responsible for the switching from Th1 to Th2 phenotype [Grewe et al 1998]. In the initiation phase, IL-4 may result in accumulation of eosinophils via upregulation of VCAM-1 on endothelial cells [Bochner et al 1994] and by acting as chemoattractant [Dubois et al 1994]. Eosinophils produce IL-12, which is known to induce a Th1 type cytokine pattern. The production of IL-12 by eosinophils is stimulated by Th2 type cytokines. Via this mechanism a switch from a Th2 to a Th1 phenotype occurs through the attraction of eosinophils and the stimulation of IL-12 production in eosinophils [Grewe et al 1998].

IL-12 has an outstanding role in skewing the Th cell responses, as other modulating factors such as IL-4, IFN-γ, IL-10, and PGE2 act by modulating either the production of IL-12 in antigen presenting cells (APCs) or the responsiveness to IL-12 in T cells [Kapsenberg et al 1998].

The most important source of IL-12 are the different APCs like Langerhans cells in the skin. They have have been implicated in the differential development of a Th1 or Th2 cytokine pattern [Kapsenberg et al 1998]. The polarisation of Th cell responses involves signals, particularly, during or shortly after their activation. As activation of T cells occurs through antigen presentation, the balance between Th1 and Th2 type responses is highly determined by APCs [Kapsenberg et al 1998]. It has been documented that IL-12 enhances the production of IFN-γ and the development of IFN-γ producing Th cells. In contrast PGE2 inhibits IFN-γ production by Th cells [Snijders et al 1998]. Both IL-12 and PGE2 can be produced by APCs. The ratio between secreted PGE2 and IL-12 may for a large part be responsible for a differential Th cell cytokine pattern. In asthma, peripheral blood monocytes were demonstrated to have a lower production of IL-12 in response to bacterial antigen as compared with control monocytes [Van der Pouw Kraan et al 1997]. This was not confirmed in AD. However, in AD an increase in PGE2 production in monocytes has been reported (Snijders 1998). Recently, it was shown that enhanced PGE2 production by monocytes in AD is not accompanied by a general rise in monocyte cytokine production indicating that AD is indeed associated with an increased PGE2/IL-12 production ratio by monocytes [Snijders

et al 1998]. This indicates that a primary abnormality in the APCs may in part be responsible for the Th2 skewing encountered in atopic diseases. Therefore, Kapsenberg et al [1998] proposed the existence of functionally distinct APC subsets with a high (type1) or a low (type 2) ability to produce IL-12 upon contact with antigen-specific T cells. Besides influencing the balance between Th1 and Th2 type cytokine patterns, APCs have been implicated in the development of tolerance. Ridge et al [1996] demonstrated that the development of tolerance is not restricted to neonatal T cells, but also occurs in adult T cells. The crucial parameters influencing the development of tolerance seem to be the dose of antigen, the type of adjuvant, and the type of APC. Whereas professional APCs activate neonatal and adult T cells, APCs lacking costimulatory molecules result in tolerance [Ridge et al 1996].

The intake of polyunsaturated essential fatty acids (PUFAs) may be important as they are involved in the regulation of the Th1/Th2 balance. Linoleic acid (found in vegetable oils) is a precursor of PGE2 that inhibits the formation of IFN-γ, whereas eicosapentaenoic acid (found in fish) inhibits the formation of PGE2 [Black et al 1997, Black 1999]. Therefore, the intake of vegetable oils may promote Th2 type responses. Support for this hypothesis comes from an epidemiological study by von Mutius et al [1998]. They found an increase in hay fewer since the reunification in East Germany that was significantly associated to a change in intake of margarine [von Mutius et al 1998]. Other factors involved in the regulation of the Th1/Th2 balance may be infections (by directly inducing IL-12 production in APCs), superantigens, the cytokine microenvironment during antigen presentation, the antigen itself (type, concentration, and route), and genetic factors [Romagnani 1996, Umetsu et al 1997].

- Effector cells

Whereas Th subsets, by producing cytokines, have a regulating role in the allergic inflammation, effector cells like mast cells, basophils, and eosinophils are responsible for the tissue damage. An overview of the T cell cytokines controlling and activating the functional activity of the effector cells involved in the allergic inflammation is presented in Figure 2. However, this figure is a simplification of the number of mediators secreted and the network of interactions between cells.

Figure 2

Pathophysiology of the allergic inflammation
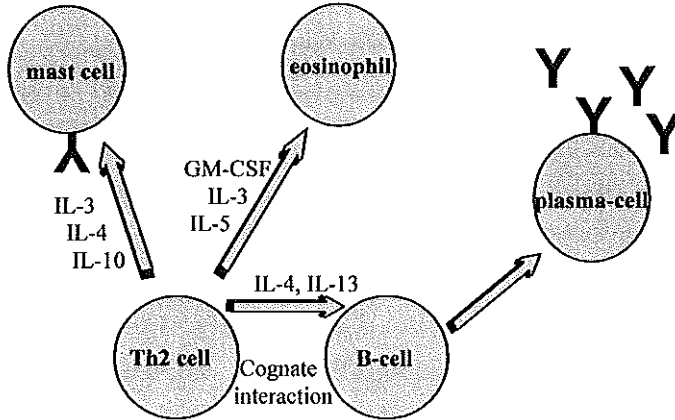
Mast cells are not only effector cells by release of a large number of mediators (histamine, tryptase, chymase, platelet-activating factor, leukotriene C4, and prostaglandin D2) but also control inflammation by secreting a large number of cytokines (IL-4, IL-5, IL-6, IL-13, TNF-α) [Church et al 1997]. Mast cells have been shown to be a major source of IL-4 themselves [Horsmanheimo et al 1994] and can also provide the B-cell help for IgE switching, the so-called cognate interaction as they express CD40L [Gauchat al 1993, Church et al 1997] Gauchat et al [1993] reported that highly purified lung mast cells and basophils were able to support IgE production by B cells in the absence of T cells. Furthermore, mast cells have been shown to be able to process and present antigens, though it is not known whether this function has any relevance in vivo [Church et al 1997].

Eosinophils are another involved cell type that contributes to the chronic allergic inflammation. They are involved in the normal response to parasitic infections, but are largely held responsible for the tissue damage occurring in allergic inflammation. The main cytotoxic proteins secreted are major basic protein (MBP), and eosinophil cationic protein (ECP). Like mast cells, eosinophils have been considered primarily as effector cells under the control of T cell cytokines. However, this turned out to be untrue, as eosinophils themselves are the source of a number of cytokines [Desreumaux 1996]. Eosinophils were found to produce IL-3, IL-5, and granulocyte monocyte-colony stimulating factor (GM-CSF)

[Desremaux et al 1996]. Interestingly, these three cytokines are the main factors in the recruitment, activation, and degranulation of eosinophils. The in vivo relevance of this observation is not known, but it suggests that once allergic inflammation is established, it may be perpetuated by such a positive feedback mechanism. Recently, eosinophils were also reported to synthesise IL-2, IL-4, IL-10, and IFN-γ [Desremaux et al 1996], which makes it difficult to understand the function of eosinophils. Another important feature of eosinophils is the presence of both low-affinity and high-affinity IgE receptors (FcεRII and FcεRII) on the surface of eosinophils [Soussi Gounni et al 1994]. The cross-linking of these IgE receptors has been shown to induce eosinophil degranulation. This means that IgE activates and induces degranulation not only in mast cells but also in eosinophils. Furthermore, the expression of FcεRI on eosinophils has been implicated in the antigen-presenting function of eosinophils. Another mechanism by which eosinophils may regulate the immune system is the production of IL-12, which induces a type 1 cytokine pattern in T cells. It was speculated that this may be the underlying mechanism for the switching from a type 2 phenotype (initiation phase of AD) to a type 1 phenotype (chronic phase of AD) in T cells [Grewe et al 1998].

In conclusion, the function of mast cells and eosinophils is more complex than initially proposed, and information has accumulated to suggest an active role in antigen presentation, regulation of a Th type 1 or 2 response, IgE switching, and various other proinflammatory processes.

Little is known on the role of other cell types present in the skin. Recently it was suggested that skin keratinocytes from patients with AD and non-atopic subjects differ in their intrinsic ability to respond to proinflammatory stimuli. Pastore et al [1998] reported that keratinocytes from uninvolved skin in AD were hyperresponsive to IFN-γ in terms of production of IL-1 alpha, GM-CSF, and TNF-alpha.

- Regulation of IgE synthesis

The presence of increased levels of IgE antibodies to common allergens is a main feature of atopic diseases. Actually, many investigators define atopy by the presence of specific IgE

Figure 3

Regulation of IgE synthesis
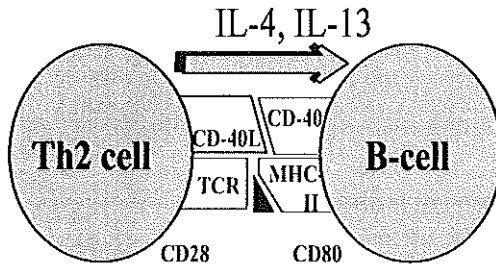
(>0.35 IU/l), a positive skin test or a positive provocation test. About 80% of the patients with AD have increased levels of IgE. As figure 3 shows, the induction of IgE synthesis requires several signals.

One signal is the presence of IL-4, which is not only produced by T cells, but also mast cells, basophils and eosinophils. With regard to IgE synthesis, IL-13 has a similar function as IL-4 [Yanagihara 1998]. The second signal is constituted by a cell to cell interaction between Tcells and B cells [Yanagihara 1998]. This process called cognate interaction involves cell surface molecules on both T cells (CD40L, T cell receptor=TCR, and CD28) and B cells (CD40, MHC class II, and CD80). Both signals together induce ε germline transcription and induce isotype switching to IgE in human B cells. The receptors for IgE can be classified into those with high affinity (FcεRI) and low affinity (FcεRII) and have primarily been localised to mast cells and basophils. However, the discovery of FcεRI on eosinophils, Langerhans cells, and monocytes changed the traditional view of IgE as just a mediator of mast cell degranulation. Besides activation and degranulation of eosinophils, IgE - FcεRI/FcεRII binding improves the antigen uptake and thereby promotes antigen presentation in different cell types (APCs, B cells, and eosinophils) [Mudde et al 1995].

- Adhesion molecules

Adhesion molecules are glycoproteins, which mediate the attachment of leucocytes to endothelial cells thereby controlling migration. Recent studies indicated that adhesion

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molecules are enhanced in acute and chronic AD lesions, in allergic skin reactions [Kyan-Aung et al 1991, Takigawa et al 1995] and even in uninvolved skin of AD patients when compared to normal control skin [Jung et al 1996]. Distinct adhesion molecules on both leucocytes and endothelial cells are involved in the different phases of the extravasation of leucocytes (Figure 4).

Figure 4

Adhesion molecules direct leukocytes to the sites of inflammation
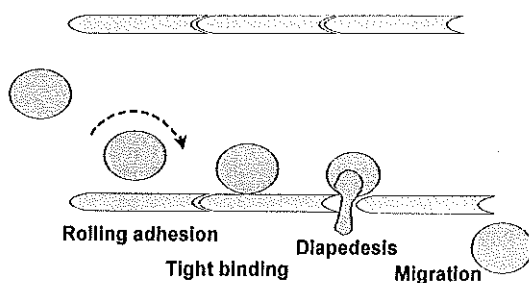
Rolling adhesion
Tight binding
Diapedesis
Migration

E-selectin and P-selectin mediate the first attachment of the leucocytes to the endothelial layer, resulting in rolling along the endothelium. This weak binding, however, promotes stronger interactions between ICAM-1 and VCAM-1 on the endothelium and their leucocyte surface ligands, resulting in tight binding [Bochner et al 1994]. The next step, diapedesis, is mediated by ICAM-1 and VCAM-1 and their receptors on leucocytes. Based on the biochemical structure adhesion molecules are classified into 4 families:

- Selectins (E-selectin, P-selectin, L-selectin)
- Vascular addressins (GlyCAM-1, CD34...)
- Integrins (LFA-1, VLA-4...)
- Immunoglobulin superfamily (ICAMs, VCAMs...)

Adhesion molecules on endothelial cells selectively bind to particular adhesion molecules on leucocytes [Bochner et al 1994]. VCAM-1 adheres to its counter-ligand VLA-4 that is expressed on eosinophils, basophils and lymphocytes but not on neutrophils [Koizumi et al

1995]. E-selectin promotes the selective migration of both CD4+ and CD8+ T cells expressing the cutaneous lymphocyte antigen (CLA) which is a skin homing factor for CD45+ RO+ memory-type T cells [Santamaria et al 1995]. The expression of adhesion molecules is controlled by locally produced cytokines and is rapidly upregulated on both endothelial cells and leucocytes upon exposure to cytokines, including interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α) and interleukin-4 (IL-4) [Bochner et al 1994]. Increased expression of ICAM-1, VCAM-1 and E-selectin has been demonstrated in skin biopsies from chronic and acute AD lesions [Takigawa et al 1995, Jung et al 1996]. Six hours after intradermal allergen injection to sensitised individuals, enhanced expression of ICAM-1 and E-selectin was found in affected skin [Kyan Aung et al 1991].

The soluble form of these adhesion molecules is present in the circulation as a result of shedding or proteolytic cleavage [Gearing et al 1993]. The level of soluble adhesion molecules in peripheral blood is an indicator of the endothelial activation [Leeuwenberg et al 1992] and may be of value as a marker of inflammation in AD [Wuthrich et al 1994, Czech et al 1996, Yamashita et al 1997]. Little is known on the function of soluble adhesion molecules. Soluble adhesion molecules may have physiological implications such as inhibition of adhesion by competitive binding. In contrast, it was also suggested that they may act in a pro-inflammatory manner by chemoattraction and by activating leucocytes (Lo 1991).

- Chemokines

Chemokines are a group of structurally related proteins, characterised by their chemotactic activity [Adams et al 1997]. However, chemokines do not only induce leucocyte migration, but are also involved in leucocyte activation, adhesion and angiogenesis [Rafeul 1997]. The term chemokines is a shortening for chemoattractant cytokines. The 28 described chemokines are classified into 3 families based on the biochemical structure. Virtually all studied blood and tissue cells secrete chemokines. As chemokines attract and activate eosinophils, mast cells, basophils and T cells they are assumed to play a crucial role in the pathophysiology of atopic diseases [Rafeul 1997]. The expression of chemokines is induced by pro-inflammatory cytokines such as IL-1, IL-2, IFN-γ, TNF-α, and lipopolysccharides

[Adams et al 1997]. In allergic diseases, eotaxin seems to have a crucial role due to the selective chemoattraction and activation of eosinophils [Adams et al 1997].

- Genetic background of atopic diseases:

It has long been known that atopic diseases run in families. A positive family history for atopy considerably increases the risk of developing atopic diseases [Dold et al 1992, Berth-Jones et al 1997]. It is generally perceived that a variety of environmental factors operate in genetically susceptible individuals. Twin studies that compare monozygotic with dizygotic twins provide data on the relative importance of genetic factors. The pairwise concordance rate for AD was 72% in monozygotic and 23% in dizygotic twin pairs (Schultz Larsen 1993). In an earlier report this was found to be 77% and 15 % in monozygotic and dizygotic twins, respectively (Schultz Larsen 1986). The high concordance rate in monozygotic twins clearly indicates that genetic factors are decisive in the development of atopic dermatitis.

Random genome searches investigating genetic linkage in affected families and candidate gene studies have established several chromosomal regions that seem to be associated with the following phenotypes: asthma (5q31-q33, 6p21.3, 11q13 and 12q14-q24), bronchial hyperresponsiveness (5q31-33), increased total IgE (5q31-q33, 11q13 and 12q14-q24), and increased specific IgE (11q13 and 14q11-q13). The candidate genes located in the above mentioned chromosomal regions are shown in Table 8.

Table 8 [After Barnes et al 1998 and Holgate 1997]:

Linked chromosomal region	Candidate genes
5q31-q33	IL-3,IL-4,IL-5, IL-9, IL-13, GM-CSF, β_2-adrenergic receptor, glucocorticoid receptor
6p21.3	Human leucocyte antigen D, tumour necrosis factor α
11q13	High affinity IgE receptor (FCεRI) , fibroblast growth factor 3
12q14-q24	IFN-γ, stem cell factor
14q11-q13	T cell receptor α (TCRα), NF-$\kappa\beta$ inhibitor

As mentioned in Table 8, all the proposed candidate genes code for proteins related to the regulation of the allergic inflammation.

The first step in finding a relationship between a phenotype and a gene is to determine its chromosomal location by genetic linkage studies. The affected sib pair analysis is particularly suitable for the analysis of atopic disorders, because a specification of the mode of inheritance is not required. In a second step, specific functional polymorphism can be related to the expression of the related phenotypes [Barnes et al 1998].

To date, more than 10 genes or chromosomal regions have been proposed to be related to asthma or atopy. However, a detailed description is beyond the scope of this thesis. Nevertheless, in AD relatively little is known on the relationship to distinct genotypes. Recently, genetic variants of mast cell chymase were claimed to be associated with AD (odds ratio 2.17), but not with asthma or allergic rhinitis [Mao et al 1996]. This association was confirmed in a subsequent study [Mao et al 1998] by the same group.

Cox et al [1998] tested several polymorphisms within Fc epsilon RI-beta for association with atopic dermatitis. They found a significant association with Fc epsilon RI-beta gene polymorphisms involving allele 2 of RsaI intron 2 (P = 0.0022) and allele 1 of RsaI exon 7 (P = 0.0036). Hershey et al [1997] reported that a specific gain-of-function mutation in the alpha subunit of the IL-4 receptor was significantly more common among patients with AD as compared with healthy non-atopic controls.

The search for genes predisposing for atopic diseases is complex due to the variable clinical phenotype, variations in the genotype between different populations, the influence of non-genetic (environmental) factors, and the involvement and interactions of presumably many major and minor genes [Scheffer 1996]. While the evidence for an association of asthma with HLA-D (6p21.3) and atopy with FCεRI (11q13) is highly suggestive, further research is needed to define the genes in the other regions and to ascertain their relevance in the expression of atopic diseases.

• Influences in utero

It is known from epidemiological studies that maternal atopy confers a higher risk to the child for developing atopic dermatitis than paternal atopy [Ruiz et al 1992]. This may be

explained by the cytokine profile to which the foetus is exposed in utero. Mothers with atopic disease during pregnancy may expose the foetus to higher concentrations of Th2 type cytokines. The mechanisms involved are not elucidated, but appeared to begin at 22 weeks of gestation [Holgate 1997]. Exposure to birch pollen after 22 weeks of gestation has been associated with T cell responses to birch pollen at birth. Furthermore maternal exposure to house dust mite during the last trimester of pregnancy was found to increase T cell responses to house dust mite in the neonate [Warner et al 1994]. However, a maternal diet during the last trimester of pregnancy was demonstrated to have no preventive effect on the development of atopic disorders [Zeiger et al 1995].

Pregnancy is associated with a predominance of Th2 type cytokines that protects the foetus from the mother's cell-mediated immunity [Warner et al 1996]. A Th1 type cytokine profile has been associated with a higher rate of abortions [Wegmann et al 1993]. This means that the foetus is exposed to Th2 type cytokines in utero. The placenta plays an important role by transporting antigens to the foetus and by maintaining a Th2 type cytokine environment. However, only some of the children develop persistent Th2 type responses. It was suggested that the foetus counteracts the placental Th2 environment by maintaining a Th1 biased environment and that only those children develop persistent Th2 responses whose PBMCs are not able to produce enough IFN-γ [Warner et al 1996]. In agreement with this hypothesis Tang et al [1994] have shown that neonates who subsequently developed a positive skin prick test (cow's milk, egg, peanut, house dust mite, cat, dog) were characterised by a reduced IFN-γ secretion at birth. Recently, it was demonstrated that low-level IgE responses to food and inhalant allergens in the first months of life were present in both atopic and non-atopic children [Prescott et al 1999]. Whereas in non-atopic children a suppression of Th2 responses occurred, in atopic children a consolidation of Th2 responses occurred [Prescott et al 1999]. The persistence of allergen-specific Th2 responses was shown to be associated with a decreased capacity for production of IFN-γ in the neonate [Prescott et al 1999].

- Local factors

The chronic inflammation in AD is characterised by depositions of eosinophil cationic protein (ECP) and major basic protein (MBP) and a mononuclear infiltrate consisting predominantly of CD1+ cells and CD4+ memory T cells. Eosinophils are present particularly in acute lesions of AD [Takigawa et al 1995]. The selective accumulation of these inflammatory cells is mediated by adhesion molecules and chemokines [Bochner et al 1994, Adams et al 1997].

At present, basic research is strongly focused on the immunological changes in AD while local factors receive little attention. There is no doubt that a systemic aberration in the immune system is a major pathogenic factor in AD. However, some phenomena cannot be explained by current concepts of a systemic deviation in the immune system.

If local factors would not be relevant, AD lesions would be present in the same intensity on the whole body. However, lesions of AD are normally restricted to predilection sites that vary with age. In infants, the diaper region is typically spared from lesions which may be the result of reduced exposure to irritants or allergens. Another possibility is that increased skin moisture has a protective effect. In line with the last hypothesis, Eberlein-Konig et al [1996] demonstrated that after only three hours of low air humidity (30%) a significant increase in skin roughness occurred in patients with AD, but not in normal controls. Such dry skin is a characteristic feature of AD and has a reduced barrier function. Dry skin may increase the risk of active lesions. The skin barrier function has been shown to be compromised even in clinically uninvolved skin, which may result in higher penetration rates of irritants and allergens. It has been suggested that an abnormality in stratum corneum lipids (ceramidase) or proteins is the reason for the defective skin barrier function. Seguchi et al [1996] demonstrated that in non-lesional skin of patients with AD, the amount of the epidermal protein filaggrin was only 32% of that present in normal controls. As filaggrin is thought to be the precursor protein of the emollient factors in the stratum corneum, a decrease in filaggrin may be partially responsible for the dry skin in AD [Seguchi et al 1996].

Exposure to hard water has recently been suggested to increase the risk of AD in children. In a recent study involving primary and secondary schoolchildren from Nottinghamshire, both

one year period prevalence and lifetime prevalence of AD in primary schoolchildren were significantly associated with domestic water hardness [Mc Nally et al 1998].

Such locally active genetic and environmental factors may determine wether the systemic aberration in the immune system manifests in the skin.

- The Role of infections

Infections have a complex role in the regulation of the immune system and there are indications that micro-organisms can either induce or prevent the development of atopy [Holgate 1997].

Infections in early life. Infections have been documented to influence the balance between Th1 and Th2 type cytokines. From an immunological point of view, infections are associated with the production of IFN-γ, IL-12 and IL-18 [Holgate et al 1997]. Such a cytokine profile decreases the chance for allergen sensitisation and may therefore be protective [Holgate 1997, Martinez et al 1994]. The postnatal period may be crucial in determining the pattern of T cell reactivity in adulthood (Holt et al 1997). The Th2 skewing observed in neonates is probably a prolongation of the phenomenon that fetal responses are consitutively shifted towards a Th2 type cytokine pattern [Warner et al 1996, Wegmann et al 1993]. As a result newborns have low level IgE responses to food and inhalant allergens and weakly primed Th2 responses which are modulated by environmental influences [Holt 1997, Prescott et al 1999]. However, it is not known why some children continue to have a Th2 type response to allergens, while others develop tolerance or a Th1 type response to allergens [Holt 1997]. The abilty to produce IFNγ at birth was found to be lowest in children with a genetic background of atopy [Liao et al 1996], and those who will develop atopy later [Tang et al 1994]. Infections early in life, alter the microenvironment of T cells, at a crucial moment when many allergens are first presented to T cells of mainly the Th0 type. In a murine model oral tolerance to ovalbumine was enhanced by concomitant administration of bacterial lipopolysaccharide (LPS). LPS is found on most enterobacteria, and it was hypothesized that a reduced colonisation by enterobacteria may lack the stimulus to support a switch from the physiological Th2 type response in early life to a predominantly Th1 type response to allergens in non-atopic individuals [Wold et al 1998]. In line with this hypothesis

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several studies demonstrated a delayed and different colonisation of the gastrointestinal tract in babies from Western countries as compared to babies from developing countries [Wold et al 1998].

The increased use of antibiotics and vaccinations, the smaller family size, and the higher socio-economic standard has led to a decline in infections which may favour a Th2 type cytokine pattern associated with atopic diseases. Epidemiological studies seem to support this hypothesis as the risk for atopic diseases is negatively correlated with the number of older siblings [Olesen et al 1997, Strachan et al 1997] and positively correlated with socio-economic standard [Golding et al 1987, Strachan et al 1997, Williams et al 1994]. Established atopic disease was demonstrated to improve upon helminth infections [Turton 1976]. Furthermore, certain viral and bacterial infections (measles and tuberculosis) were reported to decrease the risk for atopic diseases. Fujimura et al [1997] found a conversion of a Th2 cytokine pattern towards a Th1 cytokine pattern after varicella-zoster infection in patients with AD. However, this conclusion was drawn from 3 patients only. Furthermore, AD does not always improve by varicella infection, but may exacerbate as well.

Three studies are often cited to support the hypothesis of a protective role of infections on the development of atopic diseases.

1) In Japanese children an inverse relationship between delayed hypersensitivity to Mycobacterium tuberculosis and several features of atopy was reported by Shirakawa et al [1997]. They concluded that a decline in TBC infection may increase the risk of atopic diseases in a population.

2) African children who had been vaccinated with measles virus had a significantly higher risk for a positive skin prick test to house dust mite as compared with unvaccinated children who had had measles infection [Shaheen et al 1997].

3) In Italian military students, hepatitis-A seropositivity was less common among atopic than among non-atopic individuals [Matricardi et al 1997].

However, these studies were observational and Alm et al [1997] in a more experimental approach could not confirm the previous finding by Shirakawa et al [1997] on the risk of BCG vaccination for the development of atopy in children. The outcome of the study by Shirakawa et al [1997] may also be interpreted as a result of a depressed delayed-type hypersensitivity that is a typical feature in atopic individuals. Matricardi et al measured

seropositivity for hepatitis A, which is a marker of poor hygiene in general. However, this group may differ in many other relevant risk factors for atopic disease from the hepatitis-A seronegative group.

To date, there is no direct evidence that infections in early life have been more frequent in those individuals who become atopic than in those who remain non-atopic. Furthermore, infections do not simply promote a Th1 type response. Often Th1 type cytokines are produced in the early phase of infections and Th2 type cytokines later.

Thus, the role of infections in early life remains a controversial issue.

Staphylococcus aureus colonisation. Colonisation by Staphylococcus aureus (S. aureus) is a common feature in patients with AD and systemic or topical treatment with antimicrobial agents has been demonstrated to improve AD. Both observations point to a pathogenic role of S. aureus in AD. Colonisation has been demonstrated to be present in 93% of children with AD, but in only 37% of age-matched controls [Hoeger et al 1992]. Exotoxins were found to be produced by 40-50% of the S. aureus isolates from children with AD]Kemp et al 1996, Hoeger et al 1992]. Interestingly, an improvement of AD by topical antibiotics was observed even in the absence of clinically evident impetiginisation [Lever et al 1988].

Relevance for the pathophysiology of AD has been suggested by several different mechanisms. Leung et al [1993] found significant serum levels of IgE to staphylococcal exotoxins (SEA, SEB, TSST) in 57% of the patients with AD [Leung 1993]. They concluded that the toxins of S. aureus may exacerbate AD via an activation of Fc epsilon-receptor bearing cells.

Another possible mechanism is based on the ability of S. aureus exotoxins to stimulate a large proportion of T cells via interaction with the $V\beta$ region of the T cell receptor and MHC class II molecules [Kemp et al 1996]. This activation of T cells is not dependent on recognition of a specific antigen binding site on the T cell receptor and results in a stimulation of about 30% of the T cell population [Kemp et al 1996]. Such an ongoing stimulation of T cells may perpetuate inflammatory reactions in lesional skin.

Furthermore, S. aureus protein A has the capacity to directly stimulate B-cell proliferation. Recently, fundamentally different responses to S. aureus and its toxins have been described between patients with AD and normal controls [Campbell et al 1997]. They observed a significantly decreased production of IFN-γ and increased production of IL-4 in response to

S. aureus as compared with normal controls. They concluded that impaired IFN-γ production to S. aureus in vivo may result in failure to eradicate S. aureus from skin [Campbell et al 1997].

Taken together, these data indicate that the stimulation of the immune system by S. aureus and its toxins in individuals with an "atopic aberration" in the immune system may be responsible for the clinically observed association between S. aureus and AD.

Socio-economic impact

Atopic dermatitis (AD) is often perceived as a mild and temporary disease with minor impact on health status and psychosocial well being. This may be true in some of the children with mild AD, but it is certainly not true in moderate to severe AD. A recent Australian study on family impact and financial cost of AD using the questionnaire of Stein et al [1980] which had been used to assess the impact of conditions such as spina bifida, ventilator dependence and post-traumatic brain injury. The authors [Su et al 1997] concluded that childhood atopic eczema had a profound impact on the social, personal, emotional, and financial perspectives of families. Remarkably, even in mild AD, the impact on the family was comparable to that for children with insulin-dependent diabetes mellitus [Su et al 1997].

Several factors related to AD contribute to the impact on child and family.

- *Chronically relapsing disease:* The unpredictable course of the disease with ups and downs, exacerbations and infections can frustrate both parents and physicians. Unfortunately, desperation is the symptom of chronic skin disease that is hardest to treat. Parents may loose confidence in regular medicine and end up with considerable costs for alternative medicine.

- *Pruritus:* Itching is often reported by the parents to be the worst feature of AD. Itching and scratching go together. Parents, whose child scratches until bleeding wounds emerge, often report a feeling of helplessness. However, some parents demand the impossible - not to scratch. In those cases scratching may become the subject of a power struggle between parents and child. Usually, itching is most prominent in the evening and at night. In infants this results in repeated awakening, crying and sleep loss by the parents, whereas older children may experience drowsiness and lack of concentration during the day.

- *Sleep disturbances:* Dahl et al [1995] demonstrated that children with AD have several sleep disturbances compared to healthy subjects: greater difficulty in falling asleep, frequent night waking, less total sleep, and greater difficulty in waking up for school. The severity of AD was moderately correlated with sleep problems and with daytime

behaviours. Difficulty falling asleep and night waking correlated with problems in daytime behaviour and discipline.

- *Behavioural disturbance:* Parents often report that their children with eczema display hyperactive symptoms. A recent study investigating 50 children with AD and 50 children with diagnosed attention deficit hyperactivity disorder (ADHD) demonstrated a higher incidence of atopic symptoms and family history of atopy in children with diagnosed ADHD [Baron et al 1998]. Furthermore, they found a higher incidence of hyperactivity symptoms in children with atopic eczema than in the general population. The authors identified 24% of the children with AD as having hyperactivity scores (Connors parents rating scale) equivalent to those seen in ADHD. The higher prevalence of behavioural problems is confirmed by Hashiro et al [1998] who reported that patients with AD were significantly more often depressive and scored higher for state anxiety than the normal control group.

- *Cosmetic aspects:* The skin is the outer layer of our self-image. Decreased self-esteem can be the consequence of disfiguring eczematous skin lesions on the face or the hands. In this context the attitude of parents and teachers towards these skin lesions is crucial. Especially, oozing and infected lesions may prevent even parents from having regular skin contact with their baby. In daily life, normal undertaking, like shaking hands, can be painful and embarrassing.

- *Therapy:* The topical therapy in AD is rather time-consuming and can be painful due to mechanical irritation when applying the ointment. Some of the children refuse the treatment that may result in a power struggle between child and parents.

- *Risk for asthma:* It is documented that about 40% of the children with AD will develop asthma, whereas about 30% will develop allergic rhinitis [ETAC 1998, Bergmann et al 1998]. The risk for asthma is further increased in case of severe AD or additional food allergy (especially egg) [Guijet et al].

- *Food allergy:* It is estimated that about 20% of the young children with AD have food allergy. Since it is a general belief that food allergy is responsible for AD, food allergy may be assumed unjustified, and subsequently treated with a diet. In this way the child

may be put on a strict elimination diet without indication, which imposes an extra burden and, in extreme cases, a danger to the child's health and well-being.

- *Financial costs:* Financial implications of AD have been largely neglected, with only a few relevant publications in recent years [Herd et al 1996, Lapidus et al 1993, Su et al 1997]. Recent data from Australia suggest that direct financial costs in moderate to severe AD are substantially higher than those for the average child with asthma [Su et al 1997]. The annual cost to the community in mild, moderate and severe AD was estimated to be Aus$ 453 (fl 634,-), 1523 (fl 2132,-), and 2912 (fl 4076,-) per child, respectively. In the same study the annual personal cost of managing mild, moderate and severe AD was estimated to be Aus$ 330 (fl 462,-), 818 (fl 1145,-) and 1255 (fl 1757,-) per child, respectively. In another recent study from the U.K., the mean cost of AD over a 2-month period was found be £25.90 (fl 85.5,-) to the patient and £ 16.20 (fl 53.5,-) to the health service. The corresponding data for patients with severe AD were £325 (fl1073,-) to the patient and £415 (fl 1370,-) to the health service. Although these data cannot easily be extrapolated to other countries, it can be concluded that AD imposes a considerable financial burden to the family and the community.

Recently, standardised questionnaires have been developed to measure the psychosocial impact of AD on the child and the family. The impairment of the child's quality of life can be assessed by using the Children's Dermatology Life Quality index (CDLQI), while the impact of childhood AD on the family can be assessed by using the Dermatitis Family Impact questionnaire. These questionnaires will help to elucidate the impact of the disease and the efficacy of treatment in terms of psychosocial well-being. In conclusion there is considerable impact on the child and the family.

The impact of this disease is best illustrated by the experience of a patient with AD:

"For me, having AD is like having to wear a coat that feels rough, is the wrong color, is stiff where it should bend, and is unattractive to everybody. After several years of wearing this thing, you grow to hate it, yourself, and anybody who tries to make you like it."

Cited from: Sometimes the skin is only the top layer of the problem (Irene A. Crosby)

Atopic syndrome – other manifestation

The atopic syndrome includes AD, allergic rhinitis and asthma. The link between these three atopic diseases is evident in epidemiology and pathophysiology. Food allergy has been suggested as one other manifestation of the atopic syndrome. The presence of one atopic disease indicates an increased risk for the other atopic diseases. Usually, food allergy and AD are the first manifestation of the atopic syndrome, while asthma and allergic rhinitis develop later. Children with atopic dermatitis have 40 % risk to develop asthma and about 30 % risk to develop allergic rhinitis. Epidemiological studies of the past decades have demonstrated an increase in the prevalence of all atopic diseases [Ninan et al 1992, Whincup et al 1993, Burr et al 1989]. The underlying pathophysiological mechanisms are similar in AD, asthma, and allergic rhinitis as is evidenced by the inflammatory cells involved (Th cells, mast cells, and eosinophils), the inflammatory mediators involved (IL-3, IL-4, IL-5, IL-13, GM-CSF, and RANTES), tissue hyperreactivity, elevated polyclonal IgE, and the allergen-specific IgE responses. From a pharmacological point of view the link is apparent, as in all atopic diseases emphasis is put on an anti-inflammatory approach using corticosteroids. The clinical manifestations of atopy – the atopic diseases – are very variable and depend on a combination and interaction of genetic and environmental factors.

Asthma

Recent definitions of the disease concentrate on a combination of airway obstruction, airway inflammation, and airway reactivity to various stimuli. The diagnosis of asthma usually relies on a combination of a history of suggestive symptoms (wheeze, cough, dyspnoea, chest tightness), airway liability (variable airflow obstruction with > 15% change in flow rate), and increased bronchoconstrictor response to histamine or methacholine [Sears 1997]. Wheezing is a typical symptom of asthma. However, particularly in young children it is evident that not all children with asthma wheeze, and not all wheeze is asthma. Among children younger than 3 years of age there seem to be two forms of wheezing. Those with transient wheezing were shown to have constitutionally smaller airways, whereas those with persistent attacks of wheezing were characterised by manifestations of atopy [Martinez et al 1995]. The prevalence of asthma has increased during the past 3 decades and is estimated to be 5-15% in

western countries [Annesi et al 1995, Anderson et al 1994, Strachan et al 1994, Luyt et al 1993, Sennhauser et al 1995, Dold et al 1992].

Allergic rhinitis

Criteria based on consensus, for the definition or diagnosis of allergic rhinitis are lacking. Allergic rhinitis is characterised by sneezing, rhinorrhoea, and nasal blockade. Seasonal allergic rhinitis is frequently accompanied by conjunctivitis and can easily be identified, while allergic perennial rhinitis is difficult to differentiate from non-allergic rhinitis (vasomotor rhinitis). The prevalence of allergic rhinitis is low in children under the age of 5 years, but increases rapidly thereafter and reaches a peak in adolescence [Sibbald et al 1991]. From various studies it was estimated that the prevalence of allergic rhinitis approaches 20% in the general population [Dold et al 1992, Ninan et al 1992].

Atopy

The common denominator of atopic diseases is atopy. However, what exactly is atopy? Allready several decades ago Ingram complained that "Atopy means exactly nothing except in the mind of Coca and Cooke". In the literature there is a striking variety of different meanings and definitions associated with the term "atopy". The question arises on the sense of a term that causes confusion and uncertainty.

Coca and Cooke introduced the term atopy in 1923 [Coca 1923]. Atopy is derived from the Greek words a(no) topos(place). Initially atopy was meant to describe the inherited tendency to develop immediate-type hypersensitivity reactions to common antigens. Furthermore they found an association with an increased liability to form "reagins" (later named IgE antibodies). However, atopic diseases are not just immediate-type hypersensitivity reactions, and some of the individuals with AD or asthma have no evidence of underlying IgE mediated mechanisms (20 % in AD). Should those be given another name like intrinsic AD in analogy to intrinsic asthma? Is such a classification useful, since there is no difference in terms of clinical symptoms? On the other hand, the pathophysiology of AD may be different in those with and without evidence of IgE mediated mechanisms. Therefore, studies on the pathophysiology of AD should consider this different pathogenetic constellation. In 1973 Pepys defined atopy as "that form of immunological reactivity of the subject in which reaginic antibody is readily produced in response to ordinary exposure to common allergens

of the subject's environment" [Pepys 1975]. A similar definition is given in the European Allergy White Paper [Aas et al 1997], describing atopy as the hereditary predisposition to produce elevated concentrations of IgE specific to common allergens. Both definitions do not require the presence of a clinical expression of atopy and differ from the definition used by many physicians describing atopy as the hereditary predisposition to develop allergic diseases including asthma, allergic rhinitis and AD. Moreover, there are individuals with positive skin prick tests and/or specific IgE to common allergens, without having had any atopic disease. We do not know how these patients differ from the ones with clinical manifestation of atopy. Advances in the understanding of relevant genes in atopic diseases may show a specific atopic genotype associated with the regulation of IgE synthesis, and distinct organ-specific genes, associated with the target organ affected. Patients with specific IgE to common allergens without clinical symptoms may lack the distinct organ-specific genes.

Thus, the definition of atopy is controversial, and so is the diagnosis of atopy. One limitation of the diagnosis of atopy is that it does not correspond to the definition of atopy. Since most definitions of atopy describe a predisposition, strictly speaking, only genetic screening can detect atopy while the presence of total IgE, specific IgE, or a positive skin test is the net result of both genetic predisposition and environmental influences. Most authors diagnose atopy as the presence of one or more of the following features: - elevated total IgE - specific IgE – positive skin prick test – positive provocation test. The agreement between these tests is not very high and therefore a study in which atopy is diagnosed by the presence of total IgE will have a different study population than that diagnosed by skin prick test or provocation test. These differences in study population may influence the outcomes of studies.

The ongoing discussion on the definition of atopy [Rocken et al 1998, Lilja et al 1998] underscores the need for unitary criteria for definition and diagnosis of atopy.

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Chapter 1

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Chapter 2

Aims of the study

Aims of the study

The studies described in this thesis were performed in order to improve the evaluation and therapy of atopic dermatitis (AD) in childhood.

In order to study AD, a reproducible and validated scoring system for the clinical evaluation of the severity of AD is necessary. At present, the SCOring Atopic Dermatitis (SCORAD) index is the best validated clinically based system. We used the objective SCORAD which is based on the SCORAD index without subjective scoring. However, the objective SCORAD is too time-consuming for daily practice. Therefore, in *chapter 3* we evaluate a simplified scoring system, the Tree Item Severity (TIS) score, for measuring the severity of disease. Furthermore, the objective SCORAD is validated regarding its inter-observer variability in physicians working in the same institute.

Another possibility to measure the severity of disease and the outcome of therapy is the assessment of biological markers. Candidates for such markers are the soluble forms of adhesion molecules. Adhesion molecules are up-regulated in inflammatory tissues and have a crucial role in controlling the migration of leucocytes into target organs. The concentration of soluble adhesion molecules in vitro was shown to reflect the expression of adhesion molecules on the endothelial cells. Therefore, soluble adhesion molecules such as E-selectin may be used as an indicator of inflammation in the skin. In *chapter 4* we investigate this option by studying the relationship between the severity of AD as assessed by objective SCORAD and the levels of markers of inflammation (soluble adhesion molecules, eosinophil cationic protein), markers of sensitisation (total IgE, specific IgE) and the production of IL-4 and interferon-γ by peripheral blood mononuclear cells.

Studies aimed to evaluate the efficacy of treatment in moderate to severe AD by using a clinically based scoring system are described in *chapter 6*. Different aspects of therapy in children with AD are summarized in a state of the art article. A topical corticosteroid of the so-called 4th generation (fluticasone propionate 0.05% cream) is investigated to assess its suggested improved benefit / risk ratio. In children with AD, who would especially benefit from such a treatment, published studies were lacking. Therefore, we investigate the efficacy and safety of fluticasone propionate 0.05% cream in children with moderate active atopic dermatitis as assessed by objective SCORAD. However, in

severe refractory disease other approaches are required. For this purpose we propose and study a modified protocol for the "wet wrap" therapy in children with severe AD. Basically, the wet wrap therapy is an occlusive treatment with corticosteroids. Such a therapy was associated with major side-effects in the past. Therefore, the use of a corticosteroid with improved benefit / risk ratio may be a major improvement for the wet wrap therapy. Whereas in the literature a high rate of systemic side-effects is reported, our initial results with the wet wrap therapy using dilutions of fluticasone propionate 0.05% cream were promising and prompted us to investigate the efficicacy and the safety of our modified wet wrap protocol.

To further evaluate and increase the safety of the wet wrap treatment, we study daily serum cortisol using different concentrations of the corticosteroid. The aim of this study is to extend our knowledge on optimal corticosteroid concentration and the optimal duration of daily Wet Wrap treatment with fluticasone propionate.

Chapter 3

Scoring the severity of atopic dermatitis

Scoring the severity of atopic dermatitis: Three item severity (TIS) score as a rough sytem for daily practice and as a prescreening tool for studies.

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Scoring the Severity of Atopic Dermatitis: Three Item Severity Score as a Rough System for Daily Practice and as a Prescreening Tool for Studies

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Abstract

Different scoring systems have been developed to determine the severity of atopic dermatitis. The SCORAD (SCORing Atopic Dermatitis), one of the best validated systems, is suited for clinical trials, but is too complicated and time consuming for routine clinical use. The TIS score (Three Item Severity score), a simplified system, is based on the evaluation of erythema, oedema/papulation and excoriation on a scale from 0 to 3. In order to determine the value of the TIS score we conducted a prospective study in 126 children with mild to severe atopic dermatitis. Both the TIS score and the SCORAD were assessed by trained investigators. Interobserver agreement was investigated in 20 children by comparing the independently performed scores of three investigators. A positive correlation was found between the TIS score and the SCORAD (Rank Spearman $r_s = 0.86$; $p < 0.0005$). The item which correlated best with the SCORAD was excoriation ($r_s = 0.72$; $p < 0.0005$) followed by oedema/papulations ($r_s = 0.66$; $p < 0.0005$). Interobserver agreement which was calculated by Cohen's kappa (κ) was 'excellent' for SCORAD ($\kappa = 0.82$; $p < 0.001$) and 'fair' for TIS score ($\kappa = 0.58$; $p < 0.01$). We conclude that the TIS score is a rough, though reliable and simple system for scoring atopic dermatitis. It is particularly suitable in general practice, for routine clinical use and for screening purposes in clinical trials. For research purposes, the objective SCORAD offers a more detailed and comprehensive assessment.

Introduction

Atopic dermatitis is a common chronically relapsing skin disease affecting 8.9–20.4% of those born after 1970 (1). Patients with atopic dermatitis (AD) account for about 30% of dermatological consultations in general practice (2). Although guidelines give a good framework in managing AD (3), the chronically relapsing course may disappoint not only the patient, but also the physician (4). In the follow-up of such a fluctuating disease a more informative way of recording than "the patient seems better or worse" is necessary. Therefore assessing the severity of AD as objectively and reproducible as possible is extremely important not only for research purposes but also in clinical practice. However, a best scoring system for all purposes is not available (5). The choice of the system will depend on whether it is used for clinical trials, clinical routine assessment or at busy general practice. A system which is intended for research should be sufficiently discriminating and comprehensive to cover the various clinical manifestations of AD. As a result, such a scoring system will be complicated and rather time-consuming. By way of contrast, simplicity and time spent for scoring are crucial issues in a scoring system for daily routine use.

Many different scoring systems have been proposed for assessing the severity of AD (1). These systems are based on the evaluation of one or more of the following items: 1) extent, 2) a selection of intensity items, 3) subjective signs (pruritus, sleep loss) and 4) history of eczema.

For research purposes in atopic dermatitis, systems have been described by Hanifin (6), Bahmer et al. (ADASI) (7), Sowden et al. (Leicester score) (8) and Harper et al. (9). The lack of standardization prompted work groups to achieve a consensus on how to score AD. In the U.K., the Joint Workshop on Management of Atopic Eczema has recommended the Leicester system (8) for research purposes while in the the United States the 'Eczema area and severity index' (EASI) has been proposed (Clinical Dermatology 2000, Singapore). Both systems rely on the recording of different signs at different defined body sites.

In Europe, the European Task Force on Atopic Dermatitis has developed and evaluated a composite severity index based on a broad consensus by dermatologists. The resulting SCORAD index (10) consisted of information on the extent, the intensity and subjective symptoms. The SCORAD index has been used in several immunological and clinical trials

since then. The objective part of the SCORAD (extent, intensity) has been further validated with regards to the inter-observer variability in two studies with patients (11) and with the aid of a pictorial atlas (12). The subjective part (pruritus, sleep loss), which appeared to be a cause of large variations, has been scraped by the work group except for the follow-up of individual patients. The objective SCORAD is an excellent system for trials, but is too complicated and time-consuming for a routine clinical setting. Therefore, we have developed a simple scoring system called 'Three Item Severity (TIS) score', which is based on only three intensity items (erythema, oedema/papulation and excoriations). The aim of the study was to evaluate the TIS score in routine clinical practice and to investigate the correlation with the SCORAD.

Material and methods

Patients

One hundred and twenty-six children (mean age 3.9 years; range 4 months to 16 years) with atopic dermatitis according to Sampson (13) (younger than 2 years) and Williams et al. (14) (older than 2 years) were recruited from the outpatient unit of the Paediatric Dermatology Department at the University Hospital Rotterdam. All children with atopic dermatitis visiting our outpatient unit between July 1997 and March 1998 entered the study. The severity of the disease was evaluated using the SCORAD and the TIS score in all children. To assess inter-observer variation, twenty of these children were scored simultaneously by three different physicians: an expert dermatologist involved in the development of the SCORAD, another trained dermatologist and a trained non-dermatologist .

SCORAD

The objective SCORAD is a scoring system based on the assessment of extent and intensity in a standardized manner. The complete system is called SCORAD index (10) and also includes the assessment of subjective symptoms (pruritus, sleep loss) on a visual analogue scale. The extent of lesions is scored by applying the rule of nine after drawing the lesions on an evaluation form. The intensity is determined by grading each of the six items on a scale from 0 to 3 (erythema, oedema/papulation, oozing/crusts, excoriation, lichenification and dryness). Each item

should be scored on the most representative area for a given intensity item. Finally the total score is the sum of extent/5+7xintensity/2. Owing to this formule extent accounts for about 25% and intensity for about 75% of the total score. The range of the objective SCORAD lies between 0 and 83. Based on the objective SCORAD, the severity of AD can be classified into mild (<15), moderate (between 15 and 40) and severe (≥40) AD. The objective SCORAD reflects the modified consensus of the European Task Force on Atopic Dermatitis. Unlike the initial version of the SCORAD index (10), subjective symptoms are not included in the scoring of AD (11).

TIS score

The TIS score is the sum of three intensity items scored on a scale from 0 to 3 (erythema, oedema/papulation, excoriations). Similar to the objective SCORAD, each item should be scored on the most representative lesion. This means that different items may be scored on different sites. The range of the TIS score lies between 0 and 9.

Statistical analysis

Correlation between objective SCORAD and TIS score and between objective SCORAD and the different intensity items was calculated using the Rank Spearman's correlation. Inter-observer agreement for both objective SCORAD and TIS score was calculated as the intraclass correlation coefficient using kappa (κ). Agreement between observers was also calculated for each scoring item separately. A $\kappa \leq 0.4$ represents poor agreement; $0.75 > \kappa > 0.4$ represents fair agreement and $\kappa \geq 0.75$ represents excellent agreement (15).

Results

The investigated study population (n=126) consisted of children with mild (n=34), moderate (n=78) and severe (n=14) AD according to the objective SCORAD. A positive correlation was observed between TIS score and objective SCORAD (Rank Spearman's r_s=0.86 p<0.0005) (Fig. 1). The intensity item which correlated best with the objective SCORAD was excoriation (r_s=0.72 p<0.0005) followed by oedema/papulation (r_s=0.66 p<0.0005), oozing/crusts (r_s=0.6

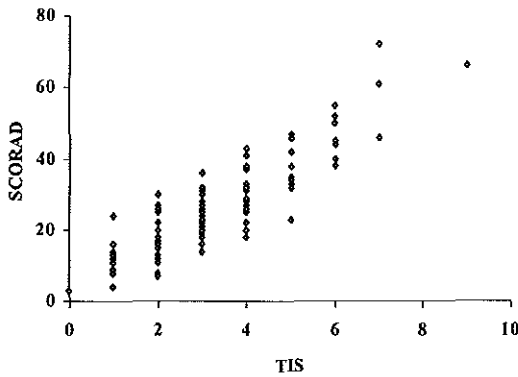**Fig. 1.** Correlation between objective SCORAD and TIS score in 126 children with AD. Rank Spearman correlation r_s=0.86, P<0.0005.
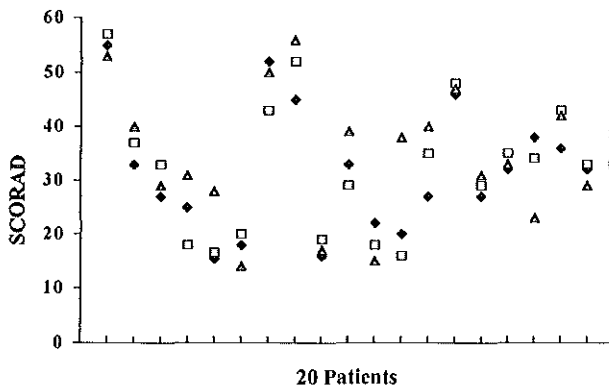

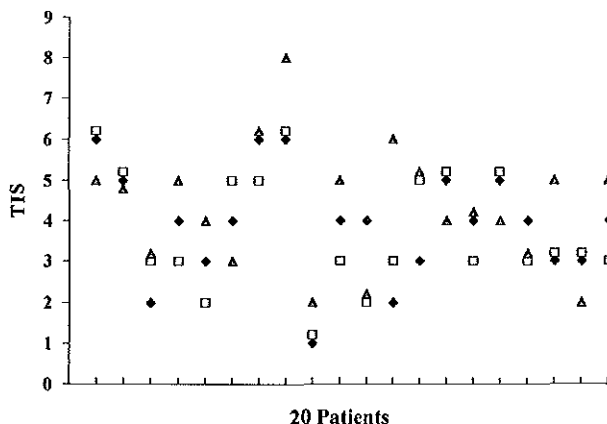**Fig. 2.** Objective SCORAD in 20 children by three different physicians (♦ expert dermatologist, ▲ dermatologist, □ non-dermatologist). Inter-observer agreement was excellent κ=0.82.

p<0.0005), erythema (r_s=0.56 p<0.0005), lichenification (r_s=0.56 p<0.0005) and dryness (r_s=0.32 p<0.0005). Extent, according to the rule of nine, also correlated well with the objective SCORAD (r_s=0.82 p<0.0005). Inter-observer agreement was assessed in 20 patients and was 'excellent' for objective SCORAD (κ=0.82; p<0.001) and 'fair' for TIS score (κ=0.58; p<0.01) (Figs. 2 and 3).

Fig. 3. TIS in 20 children by three different physicians (♦ expert dermatologist, ▲ dermatologist, ☐ non-dermatologist). Inter-observer agreement was fair κ=0.58.

Furthermore, Figs. 2 and 3 show that a trained physician (non-dermatologist) scores AD as good as a trained dermatologist.

When each scored item was calculated separately (Table I), we observed that the inter-observer agreement was the highest for extent and the lowest for oedema/papulation. Table I shows the between-patient variance, the total variance and the interobserver agreement (κ) for each of the scored items. Generally, a higher intensity of AD did not increase the variation between observers. An exception was the scoring of lichenification where the interobserver variation increased with the objective SCORAD (Rank Spearman's r_s=0.56 p<0.05).

Discussion

The TIS score is a simple scoring system for AD which is quick and easy to perform.

In the present study we observed a high correlation of the TIS score with the objective SCORAD and a fair inter-observer agreement between physicians in the TIS score.

Table I. Inter-observer agreement (kappa) in 20 children by three different physicians.

	$\sigma^2_{PATIENT}$*	σ^2_{TOTAL}†	*Kappa*‡
Objective SCORAD	115.88	141.32	0.82
TIS	1.22	2.11	0.58
Extent	627.64	679.72	0.92
Lichenification	0.9	1.19	0.76
Dryness	0.51	0.73	0.7
Oozing / crust	0.39	0.69	0.57
Excoriation	0.23	0.42	0.56
Erythema	0.24	0.45	0.52
Oedema / papulation	0.25	0.6	0.41

* $\sigma^2_{PATIENT}$ stands for the between-patient variance.

† σ^2_{TOTAL} stands for the total variance.

‡ kappa represents inter-observer agreement. A kappa ≤ 0.4 represents poor agreement, kappa between 0.4 and 0.75 represents represents fair agreement and kappa > 0.75 represents excellent agreement.

Of the six intensity items scored in the objective SCORAD, excoriation was the one that clearly correlated best with the objective SCORAD. This reflects the clinical observation regarding pruritus as a prominent symptom of AD. Next to excoriation, oedema/papulation also showed a good correlation with the objective SCORAD. This supports our choice of including these two items in the TIS score. Dryness was the only item that showed poor correlation with the objective SCORAD. While we found a high correlation between the TIS score and the objective SCORAD, it is clear from Fig. 1 that there is still so much variation left that the TIS score cannot accurately predict the objective SCORAD for all individual patients. This means that the TIS score and the objective SCORAD cannot be used interchangeably in individual patients.

In contrast to earlier studies in which considerable inter-observer variation was reported (11,16), we demonstrate excellent inter-observer agreement between the three physicians using the objective SCORAD. We believe that this is a result of both a training in scoring AD and the fact that the physicians involved already used the objective SCORAD for scoring AD in clinical routine.

A scoring system which is used in daily clinical routine should be as simple as possible which may result in a less sensitive and accurate system. During the course of time, a few systems have been developed with this in mind (16,17). Of these scoring systems, only the Basic Clinical Scoring System (BCSS) (16) is as simple and quick to perform as the TIS score. In the BCSS, the extent of AD is scored by evaluating the number of sites involved (5 sites are scored). Each site is scored 0 (no lesion) or 1 (lesion). This system was demonstrated to have an excellent inter-observer agreement, but showed poor agreement with the SCORAD index. The drawback of this system is that it presumably provides a poor reflection of therapeutic interventions. In fact, when patients with AD are treated, the involved sites will not clear completely but improve in extent and intensity without a change of the BCSS. Therefore the BCSS is probably not suited for the follow-up of patients and for evaluating the efficacy of therapy. Although sensitivity to change in individual patients has never been addressed in any study it is known from clinical practice that the items represented in the TIS score are among the first to improve under treatment. The choice of the items used in the TIS score was based on the following criteria:

1) The items should be relevant for all age groups.
2) If two items are highly correlated only one is scored.
3) The items should reflect disease severity and should be independent of other interfering factors.
4) The items should be subject to change and improve when AD improves.
5) No combination of objective signs with subjective symptoms.

Dryness, xerosis or scaling are a very characteristic feature of AD. They are used in many scoring systems but were not included in the TIS score as they largely depend on when the emollient was last applied. Lichenification which is also used in many scoring systems is not relevant in the very young children as it does not occur before the age of two years in Caucasians. Furthermore, lichenification responds rather slowly to therapy and is therefore not suited when evaluating the short-term effects of therapy. Oozing is very typical in infants, but is rare in older children. Moreover, oozing is closely linked to erythema. and is therefore already represented by erythema in the TIS score.

Subjective symptoms like pruritus or sleep-loss are strongly influenced by psychological factors and can cause large variations. Therefore, these symptoms were not included in the TIS

score. However, these symptoms remain important as an indicator of the quality of life and may serve as a separate measurement tool for follow-up (11). For a complete measurement of the quality of life, DLQI (18) for adults and CDLQI (19) for children are appropriate techniques. Results of earlier studies indicate that the objective SCORAD can also be used by non-dermatologists if they are trained to score AD (12). This is also true for the TIS score as in our study we did not find a difference between the scores by a dermatologist and a non-dermatologist as compared with those by an expert dermatologist.

In conclusion, we demonstrate that the TIS score is a reliable and simple scoring system for atopic dermatitis with a fair inter-observer agreement. It is particularly suitable for general practice, for routine clinical use and for screening purposes in clinical trials. However, for research purposes, when a sensitive method is required, the objective SCORAD offers a more detailed and comprehensive assessment of AD with an excellent inter-observer agreement.

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Chapter 4

Evaluation of immunological markers in atopic dermatitis

4.1 Disease severity and soluble E-selectin and other markers of inflammation in children with atopic dermatitis.

Brit J Dermatol 1998; 138: 431-5.

4.2 Soluble E-selectin and soluble ICAM-1 as markers of the activity of atopic dermatitis.

Submitted

4.3 Cytokine production by peripheral blood cells from children with atopic dermatitis.

Submitted

Disease severity and soluble E-selectin and other markers of inflammation in children with atopic dermatitis.

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Abstract

E-selectin, P-selectin, interstitial cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are membrane bound adhesion molecules which mediate the attachment of leucocytes to endothelial cells. These molecules are preferentially expressed on activated endothelium. The soluble form of these molecules will be present in the circulation as a result of shedding. Some of the soluble adhesion molecules have been thought to reflect disease activity in atopic dermatitis (AD). To evaluate their potential to reflect disease activity in AD, we correlated their plasma concentration with clinical severity measured by objective SCORAD (SCORing Atopic Dermatitis). Furthermore, levels of total IgE, specific IgE, and eosinophil cationic protein (ECP) were determined. SCORAD and sE-selectin levels were significantly increased in children with specific IgE for both food and inhalation allergens ($p < 0.05$). ECP consistently showed an increase with the scores of SCORAD, but no statistical significance was reached. Disease activity was significantly correlated with the plasma levels of sE-selectin ($r_s = 0.6$, $p < 0.0005$) but not with sP-selectin, sICAM-1 and sVCAM-1. This agrees with recent studies performed in adults with AD, and

supports the potential of sE-selectin as a parameter for monitoring disease activity in young children with AD.

Introduction

Although the precise pathogenesis of atopic dermatitis (AD) is still not entirely elucidated, a number of immunological features have been proposed, as markers of inflammation in AD. Total serum IgE is increased in 80% of the patients and elevated values are loosely associated with more severe disease[1]. Activated eosinophils which are increased in blood and affected skin are widely held responsible for the tissue damage in AD. One of the most important toxic proteins released from these cells, eosinophil cationic protein (ECP), has been shown to reflect disease activity[2]. Recently soluble adhesion molecules were proposed as markers of disease activity[9-14]. Membrane bound adhesion molecules are glycoproteins which mediate the attachment of leucocytes , including eosinophils, to endothelial cells thereby controlling migration. E-selectin and P-selectin mediate the first attachment of the leucocytes to the endothelial layer, resulting in rolling along the endothelium. This weak binding, however, promotes stronger interactions between ICAM-1 and VCAM-1 on the endothelium and their leucocyte surface ligands, lymphocyte function associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4), respectively[3]. Adhesion molecules are rapidly upregulated on both endothelial cells and leucocytes upon exposure to cytokines, including interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4)[3]. Increased expression of ICAM-1, VCAM-1 and E-selectin have been demonstrated in skin biopsies from chronic and acute AD lesions[4]. Six hours after intradermal allergen injection to sensitized individuals, enhanced expression of ICAM-1 and E-selectin was found in affected skin[5]. The soluble form of the adhesion molecules, sE-selectin, sP-selectin, sICAM-1 and sVCAM-1 originates from membrane bound molecules due to shedding or proteolytic cleavage[6]. The amount of soluble sE-selectin and sICAM-1 released, was found to correlate directly with the cell surface expression in vitro[7]. Thus, the levels of sICAM-1 and sE-selectin may reflect the intensity as well as the extent of the inflammation in AD. In vivo serum levels of sICAM-1

and sE-selectin have been shown to be increased in asthma[8] and AD[9]. Recently several studies reported a correlation between disease severity and the soluble adhesion molecules sICAM-1 and sE-selectin in AD[10-14]. However, such data are not yet available from children. Moreover no comparison was made between sE-selectin and ECP, the most extensively studied marker of severity in AD so far. In a pilot study we compared the levels of soluble adhesion molecules (sICAM-1, sE-selectin) in small groups of children with allergic asthma, non-allergic asthma, atopic dermatitis and healthy controls[15]. In the group with atopic dermatitis (n=10) we found a correlation between the levels of sE-selectin and severity of disease. In the present study we investigated whether and to what extent ECP, total IgE, specific IgE, sE-selectin, sP-selectin, sICAM-1 and sVCAM-1 are correlated to disease severity in children with AD.

Methods

Patients and methods

Forty children (21 boys and 19 girls; mean age: 22,3 months; range: 13 to 36 months) mainly with mild to moderate AD[16] (one child had severe AD) participated in the present study. All of the children had a positive family history for atopy and no diagnosis of asthma. Children with AD were treated with emollients, and as required with topical glucocorticosteroids, tar ointments and systemic antihistamines. None of the children was treated with systemic corticosteroids. The study including blood sampling was approved by the Medical Ethical Committee of the University Hospital Rotterdam and the Zuiderziekenhuis, and informed consent was obtained from the parents prior to participation.

Clinical scoring system

The severity of disease was determined by the modified SCORAD system[17]. accepted for publication Dermatology), which is a composite index including the assessment of two items in a standardized way: a) extent (applying the rule of nine) and b) intensity (erythema, edema/papulation, oozing/crust, excoriation, lichenification, dryness). This scoring system,

called SCORAD, reflects the modified consensus of the European Task Force on Atopic Dermatitis. Unlike the initial version of the SCORAD index[18], subjective symptoms are not included in the scoring of AD.

Determination of soluble adhesion molecules

sE-selectin and sICAM-1 were measured in plasma by ELISA as described previously[19]. Briefly, 96-wells microtitre plates were coated with monoclonal antibodies to sICAM-1 (HM.2, 3 μg/ml) or sE-selectin (ENA1, 10 μg/ml) in phosphate-buffered saline (PBS) followed by saturation with 1% bovine serum albumine. Standard or diluted samples were added in 100 μl/well. After two hours at room temperature, the plates were washed and incubated for one hour with biotin labeled anti-ICAM-1 or anti-E-selectin monoclonal antibodies. All antibodies were a kind gift of dr WA Buurman (University of Maastricht, The Netherlands). After washing streptavidin-conjugated peroxidase was added. Peroxidase activity was determined by addition of 2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS). The reaction was stopped with H_2SO_4 and read at 414 nm. The concentration of sICAM-1 and sE-selectin were determined by interpolation from the standard curve. The detection limits of these ELISA assays were 400pg/ml for sICAM-1 and 200 pg/ml for sE-selectin. Both sP-selectin and sVCAM-1 were measured by commercial ELISA's in plasma, based on a similar procedure using a commercially available test kit (R&D Systems Europe, Abbigdon, UK).

Determination of total and specific immunoglobulin E (IgE)

Total IgE was determined in the sera by chemiluminescence (Ciba Corning ACS). Specific IgE for food allergens (cow's milk, egg) and inhalation allergens (house dust mite, grass pollen, cat dander) was determined by CAP Farmacia Upjohn (Uppsala Sweden).

Data analysis

Statistical analysis was performed with SPSS/PC+, using the Mann-Whitney U test to assess differences between groups (total IgE, specific IgE for food and inhalation allergens) in SCORAD and soluble adhesion molecules. The Spearman rank correlation was used to test

monotonic relationships between SCORAD, soluble adhesion molecules, specific IgE, ECP, myeloperoxidase (MPO) and eosinophils. Statistical significance was defined as p<0.05.

Results

IgE, specific IgE, peripheral eosinophils and eosinophil cationic protein (ECP)
Total IgE levels in the sera of the children were increased above the age specific limit (<2 years: 0-29 kU/l; >2 years: 0-69 kU/l) in 23(of 39) children. These children had significantly more severe disease, as reflected in increased SCORAD, than children with normal total IgE (p<0.01). Specific IgE for food and inhalation allergens were found in 21(of 38) and 20(of 39) children, respectively. In those children with increased specific IgE to both food and inhalation allergens increased disease activity was also observed (p<0.05) (fig.1).
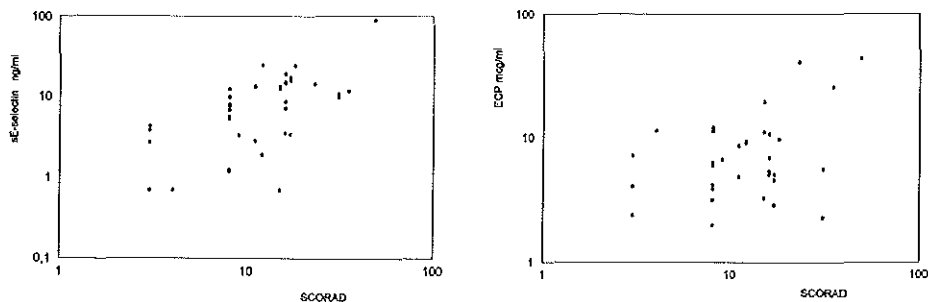
Figure 2. (a) The relation between serum ECP and clinical severity as measured by SCORAD. Rank Spearman r_s=0.254, p=0.15 (n.s.). (b) The relation between plasma levels of E-selectin and SCORAD. Rank Spearman r_s= 0.6013, p<0.0005.

Similarly a positive correlation was found between the severity of disease and the RAST class for both food allergens (r_s=0.39, $p<0.05$) and inhalation allergens (r_s=0.33, $p<0.05$). Peripheral eosinophil concentrations were found to be increased (>5%) in 7(of 37) children and correlated with disease activity (r_s=0.34, $p<0.05$). Serum levels of eosinophil cationic protein were increased in only 4 (of 34) children. These children had significantly more severe disease than children with normal levels of eosinophil cationic protein ($p<0.01$). However, when analyzing the group as a whole, no significant correlation was found between eosinophil cationic protein and disease severity (r_s=0.25, $p>0.05$) (fig.2a).

sE-selectin in plasma

The mean plasma concentration of sE-selectin was 10.9 ± 14.3 ng/ml (mean ± SD). A significant correlation was found between plasma levels of sE-selectin and the SCORAD (r_s=0.6, $p<0.0005$) (fig. 2b). Those children who had specific IgE for both food allergens and inhalation allergens had significantly higher levels of sE-selectin than those who had not ($p<0.05$) (fig. 3). Moreover sE-selectin levels correlated with the RAST class for food allergens (r_s=0.44, $p<0.01$) and inhalation allergens (r_s=0.33, $p<0.05$) respectively, but not with total IgE.

When the levels of soluble E-selectin were corrected for SCORAD, a significant ($p<0.05$) positive correlation was observed between levels of sE-selectin and age.

sICAM-1, sVCAM-1 and sP-selectin in plasma

The mean plasma concentrations of sICAM-1, sVCAM-1 and sP-selectin were respectively 253.7 ± 178.6 ng/ml, 870.5 ± 292.4 ng/ml and 109.6 ± 34.8 ng/ml (mean ± SD). A positive correlation was found between the levels of sP-selectin and sVCAM-1 ($_s$=0.45, $p<0.005$). However no significant correlations between these soluble adhesion molecules and SCORAD (sICAM-1 r_s=0.02, $p>0.05$; sVCAM-1 r_s=0.02, $p>0.05$; sP-selectin r_s=0.14, $p>0.05$) were detected.
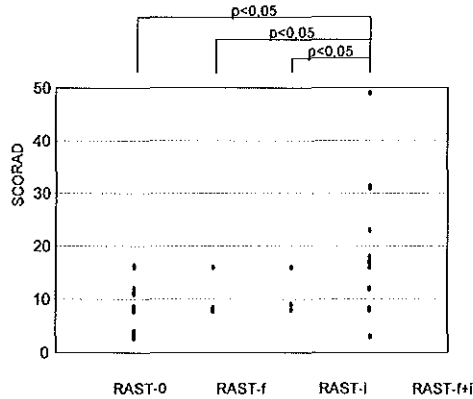
Figure 1. SCORAD in children without specific IgE (RAST negative), with specific IgE for food allergens (RAST food), inhalation allergens (RAST inhalation) and for both food and inhalation allergens (RAST food, inhalation). Specific IgE was tested for cow's milk, egg, cat dander, house dust mite and grass pollen). For statistical analysis the Mann Whitney U test was used.

Discussion

The present study shows a significant correlation between the plasma levels of sE-selectin and the clinical severity of AD (SCORAD) in children. Such a correlation could not be demonstrated between SCORAD and the other adhesion molecules sICAM-1, sVCAM-1 and sP-selectin.

This finding agrees with a recent study in adults with AD, in which disease severity was found to correlate with sE-selectin but not with sICAM-1[20]. In the same study clinical improvement was associated with a significant drop in sE-selectin levels. Other studies involving adults found a similar correlation between disease severity and sE-selectin[10,14]. Contradicting reports have been published concerning the correlation between sICAM-1 and

disease severity. Several studies reported a positive correlation of sICAM-1 with disease activity[10,11,13] ,while others did not find such a correlation[20] and one study even reported a negative correlation[21]. Our data do not confirm a clear correlation between eosinophil
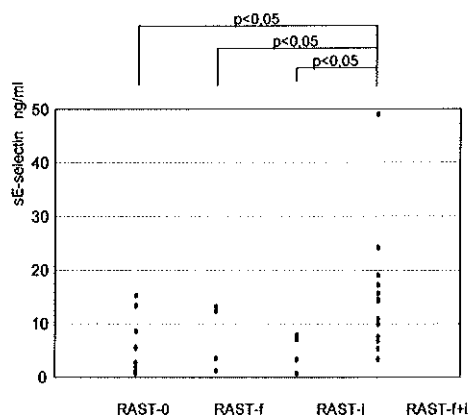

Figure 3. Soluble E-selectin in children without specific IgE (RAST negative), with specific IgE for food allergens (RAST food), inhalation allergens (RAST inhalation) and for both food and inhalation allergens (RAST food, inhalation). Specific IgE was tested for cow's milk, egg, cat dander, house dust mite and grass pollen). For statistical analysis the Mann Whitney U test was used.

cationic protein (ECP) and disease severity as was reported by others. Although those children with increased levels of ECP had significantly more severe disease, there was no correlation between levels of ECP and disease severity in the whole group of patients.

The observed discrepancies to other studies with respect to ECP and soluble ICAM-1 may be explained by different study populations and different scoring systems which were used to assess disease severity of AD. We included young children with mainly mild to moderate AD. This may be important because in acute AD exacerbations other adhesion molecules may be expressed than in mild to moderate chronic AD[22]. Furthermore weak correlations between disease severity and biological markers may only be detected when comparing mild

to severe AD.

In addition the choice of the scoring system may influence the outcomes as well. The scoring system we used (SCORAD) is based on the consensus of the European Task Force on Atopic Dermatitis. This system reflects acute and chronic components of disease activity, but shows a variability of more than 20%.

Adhesion molecules are essential not only for the adhesion of leucocytes to endothelium but also for diapedesis and chemotaxis. They selectively bind particular leucocyte types. VCAM-1 adheres to its counterligand VLA-4 which is expressed on eosinophils, basophils and lymphocytes but not on neutrophils[24]. E-selectin promotes the selective migration of both CD4^{+} and CD8^{+} T cells expressing the cutaneous lymphocyte antigen (CLA) which is a skin homing factor for CD45^{+} RO^{+} memory-type T cells[23]. Furthermore E-selectin is involved in the recruitment of neutrophils and monocytes. The expression of adhesion molecules is controlled by locally produced cytokines.

Little is known of the function of soluble adhesion molecules. It has been suggested that release

of soluble adhesion molecules in the circulation, may have physiological implications, such as inhibition of adhesion by competition. Furthermore they may act in a proinflammatory manner by chemoattraction and by activating leukocytes.

Increased levels of soluble adhesion molecules (sICAM-1, sE-selectin) have been reported in a variety of diseases including asthma[24], rheumatoid arthritis[25], lupus erythematosus[25], psoriasis[14] and certain forms of melanoma[26].

Kinetics of the expression of soluble adhesion molecules may well be of relevance in the chance to be detectable as a marker of disease. The rates of release and clearance, as well as binding, reflected by blood concentration-time curves, will partly determine the applicability of these levels to reflect a localised disease process. While no in vivo data are available, interesting differences were described in vitro between the kinetics of sICAM-1 and sE-selectin[8]. After stimulation of endothelial cells with IL-1 and TNF, sE-selectin levels were maximal after 6 to 12 hours and dropped below detection limit after 24 hours. In contrast sICAM-1 gradually increased over a period of 24 hours and remained stable for 3 days.

In vivo the expression of VCAM-1 and ICAM-1 was found to be significantly increased in

nonlesional atopic skin in comparison to the skin of normal individuals[27]. Thus, VCAM-1 and ICAM-1 were upregulated even in nonaffected skin of patients with AD while E-selectin was detected exclusively in affected skin. This indicates a higher specificity of E-selectin for affected skin and may explain why soluble E-selectin is a better indicator of disease severity. In conclusion this is the first report to show that in children with mainly mild to moderate AD, sE-selectin correlates best with the SCORAD, as an indicator of the severity of disease. Although sE-selectin is not specific for AD, it reflects disease activity and may be a reliable laboratory marker.

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Soluble E-selectin and soluble ICAM-1 levels as indicators of effective treatment in atopic dermatitis

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Abstract

Background: The expression of adhesion molecules is upregulated in the skin of atopic dermatitis (AD) patients, and the levels of the soluble adhesion molecules sE-selectin and sICAM-1 have been reported to reflect the endothelial activation in the skin of AD patients.

Objective: To investigate the relationship between symptom score and levels of sE-selectin, sICAM-1 and sVCAM-1 before and after two weeks of treatment.

Methods: Eighteen children with an exacerbation of AD were admitted and treated with corticosteroid dilutions under occlusive wet dressings (wet wrap treatment). Symptom score (objective SCORAD) and levels of sE-selectin, sICAM-1, and sVCAM-1 were assessed before and after two weeks of treatment.

Results: A significant correlation between the objective SCORAD before treatment and the level of sE-selectin ($p<0.05$), but not the level of sICAM-1 ($p=0.7$) or sVCAM-1 ($p=0.5$) were observed. The treatment resulted in a high degree of remission, which was reflected by a significant decrease in the level of sICAM-1 ($p<0.01$), whereas there was a trend in the level of sE-selectin to decrease ($p=0.08$). Most importantly, the level of sE-selectin after 2 weeks of treatment still correlated significantly with the SCORAD at the beginning ($p <0.005$).

Conclusion: Soluble E-selectin is a good marker for the severity of AD. However, it does not reflect acute changes in the symptom scores, but may be useful as a marker for the long-term activity of AD.

Introduction

Atopic dermatitis is a common chronically relapsing inflammatory skin disease affecting 8.9–20.4% of those born after 1970.[1] Research on the underlying mechanism of AD requires a reproducible system to score the activity of AD. The limitations of a clinical scoring system based on evaluation of symptoms are the need for dermatological training of the investigator, and the intra- and interobserver variability, which may account for variations of up to 60% depending on the investigator and the scoring system.[2] Another drawback of such a system is that it represents a snapshot of the activity of AD. The typical fluctuations in the severity of symptoms with spontaneous exacerbations and remissions during a short period of time may make it difficult to find the relation between symptom scores and underlying stable immune deviations. Therefore, an objective marker, which reflects a prolonged period of disease activity, may improve investigations into the pathogenic mechanisms in AD.

Different serum parameters have been reported to reflect the inflammation in AD. Total serum IgE is increased in about 80% of the patients with AD, but is only loosely associated with the severity of AD[3]. Eosinophil cationic protein (ECP - a marker for eosinophil activation), soluble IL-2 receptor and soluble CD14 (a marker for monocytes) reflect distinct components of the inflammation in AD and have been reported to correlate with the clinical severity of AD.[4-6] Recently, soluble adhesion molecules were proposed as markers of disease activity in AD.[7-13] The adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are increased in acute and chronic skin lesions in AD[14]. The serum levels of sE-selectin and sICAM-1 released from endothelial cells were observed to correlate directly with the cell surface expression in vitro.[15] Thus, the levels of sICAM-1 and sE-selectin may reflect the intensity of the inflammation as well as the extent of skin involvement in AD. We have previously reported a correlation between the level of sE-selectin and the severity of disease as measured by objective SCORAD.[16-17] Whereas a large number of studies have established the relation between symptom score and the level of sE-selectin, conflicting results have been reported on the applicability of sE-selectin for monitoring the effect of treatment.[11-13] While Kagi et al[11] reported a significant decrease in sE-selectin levels after 16 weeks of treatment with cyclosporin A (4-4.5 mg/kg) Halmerbauer et al[18] reported no significant decrease of sE-selectin after 4 days of treatment

with systemic corticosteroids. The aim of the present study was to investigate whether the levels of sE-selectin, sICAM-1 and sVCAM-1 reflect the severity of AD and the clinical improvement of AD after 2 weeks of treatment in children with severe AD.

Methods

Patients and design

Eighteen children aged 4 months to 9 years with AD[19,20] were included. All patients had AD resistant to conventional therapy as outlined in guidelines[21]. At the moment of inclusion, disease severity was severe in 16 of the patients and moderate in 2 of the patients as judged by objective SCORAD[22]. Patients were treated according to the modified wet-wrap protocol as previously described[23]. The initial wet-wrap protocol was described by Goodyear et al[24] as a treatment that results in remissions after only 3 to 5 days in severe generalized AD. Briefly, patients were admitted to the hospital for at least 1 week and treated once daily with a diluted corticosteroid (fluticasone propionate cream 0.05%) on the whole body under occlusive wet dressings (tubifast). All patients were treated according to the described protocol for 2 weeks. Other treatments for AD except antihistamines were stopped. The severity of AD was assessed by SCORAD at the beginning and at the end of the treatment.

Clinical scoring system

The severity of disease was scored using the objective SCORAD[22] which includes the assessment of two items in a standardised manner: a) extent (applying the rule of nine) and b) intensity (erythema, oedema/papulation, oozing/crust, excoriation, lichenification and dryness on a scale from 0 to 3). This scoring system reflects the modified consensus of the European Task Force on Atopic Dermatitis. Unlike the initial version called SCORAD index,[25] subjective symptoms were not included in the scoring of AD.[22,26]

Determination of sE-selectin, sICAM-1 and sVCAM-1

Serum was harvested from venous blood and stored at −20°C. Levels of sE-selectin (HBT, Uden, The Netherlands), sICAM-1 and sVCAM-1 were determined in the sera using a

commercially available ELISA kit (Bio Source Fleurus, Belgium). ELISA procedures were performed according to the manufacturer's instructions. The levels of sE-selectin, sICAM-1 and sVCAM-1 were expressed in ng/ml. The detection limits of these ELISA assays were 1.0 pg/ml for sICAM-1, 2.0 pg/ml for sVCAM-1 and 2.0 pg/ml for sE-selectin.

Safety analysis

To determine the systemic load of the topical medication, we assessed the hypothalamic-pituitary-adrenal (HPA) axis suppression. Levels of 9 a.m. serum cortisol were determined at the beginning (baseline) and at the end of the treatment period. Local side effects were assessed by visual inspection.

Statistical analysis

Statistical analysis was performed with SPSS 7.5 using the Wilcoxon rank sum test to assess differences in the levels of sE-selectin, sICAM-1 and sVCAM-1 at the beginning and at the end of the treatment. The Spearman rank correlation was used to test monotonic relationships between the SCORAD and the levels of sE-selectin, sICAM-1 and sVCAM-1. Statistical significance was defined as $p < 0.05$.
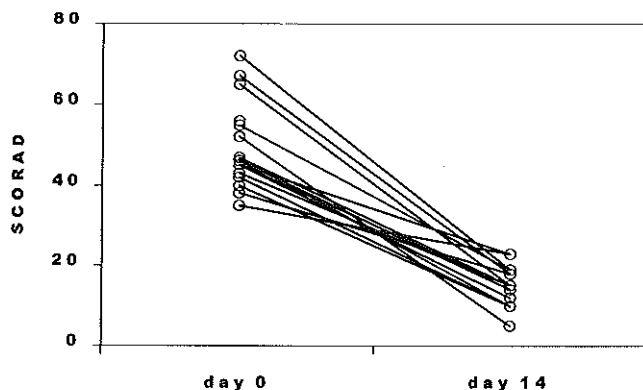

Figure 1.

Graph showing the objective SCORAD at the beginning and after 2 weeks of treatment.
The change in objective SCORAD was assessed by Wilcoxon rank sum test ($p < 0.0001$).

Results

First, we examined the efficacy and safety of the wet wrap treatment. For evaluation of the efficacy, the objective SCORAD at the beginning and after 2 weeks of the treatment were compared and are shown in *Figure 1*. There was a significant decrease in both the intensity and the extent of AD as reflected in the objective SCORAD after 2 weeks of treatment (p<0.0001). The severity of AD classified into mild, moderate and severe according to the objective SCORAD decreased in all of the children after 2 weeks of treatment *(Table I)*. One child was lost to follow-up due to personal reasons. The improvement in AD was most prominent in children with the highest objective SCORAD before treatment. For the evaluation of the safety, we assessed the levels of 9 a.m. serum cortisol at the beginning and after 2 weeks of the treatment. Serum cortisol levels were temporarily below the normal range (0.2-0.8 U/l) in 3 out of 18 children after 2 weeks, indicating a suppression of the HPA axis. These results demonstrated that in our study population the wet wrap was highly effective in reducing the severity of AD.

Table I

Classification of children according to diseases severity

before and after wet wrap treatment

	Before treatment n=18	*After treatment N=17*
Mild AD	0	11
Moderate AD	2	6
Severe AD	16	0

Mild AD: objective SCORAD □15

Moderate AD: objective SCORAD 15-40

Severe AD: objective SCORAD □40

To investigate whether the serum levels of soluble adhesion molecules reflected the improvement of AD, we compared the levels of sE-selectin, sICAM-1 and sVCAM-1 at the beginning and after 2 weeks of the treatment. Although there was a trend for the levels of sE-selectin to decrease after treatment, statistical significance was not reached in this period (p=0.08) *(Figure 2A)*. The serum levels of sICAM-1 *(Figure 2B)* were significantly reduced after treatment (p<0.01), whereas the levels of sVCAM-1 *(Figure 2C)* did not change significantly after treatment (p=0.5). These results indicated that the marked improvement in AD was only reflected by the decreased level of sICAM-1, but not by the levels of sE-selectin or sVCAM-1.

Next, we investigated whether the serum level of soluble adhesion molecules reflected the severity of AD at the time of blood sampling. The levels of sE-selectin, sICAM-1, and sVCAM-1 were compared with the objective SCORAD. A significant correlation between the objective SCORAD and the level of sE-selectin was noted (r=0.54; p<0.05) *(Figure 3A)*, while no correlation was observed with the levels of sICAM-1 (r=-0.12; p=0.7) or sVCAM-1 (r=-0.17; p=0.5). After 2 weeks of treatment, a negative relationship between the level of sE-selectin and objective SCORAD was noted *(Figure 3B)*. This resulted from the observed higher efficacy of the treatment in children with more severe AD and higher levels of sE-selectin at the beginning as compared to the children with less severe AD at the beginning (Rank Spearman correlation between objective SCORAD at the beginning and the decrease in SCORAD during treatment: r=0.79; p<0.0005).

Figure 2.
Graph showing the levels of soluble adhesion molecules at the beginning and after 2 weeks of treatment. A) sE-selectin did not change significantly (p=0.08). B) sICAM-1 decreased significantly (p<0.01). C) sVCAM-1 did not change significantly (p=0.5).
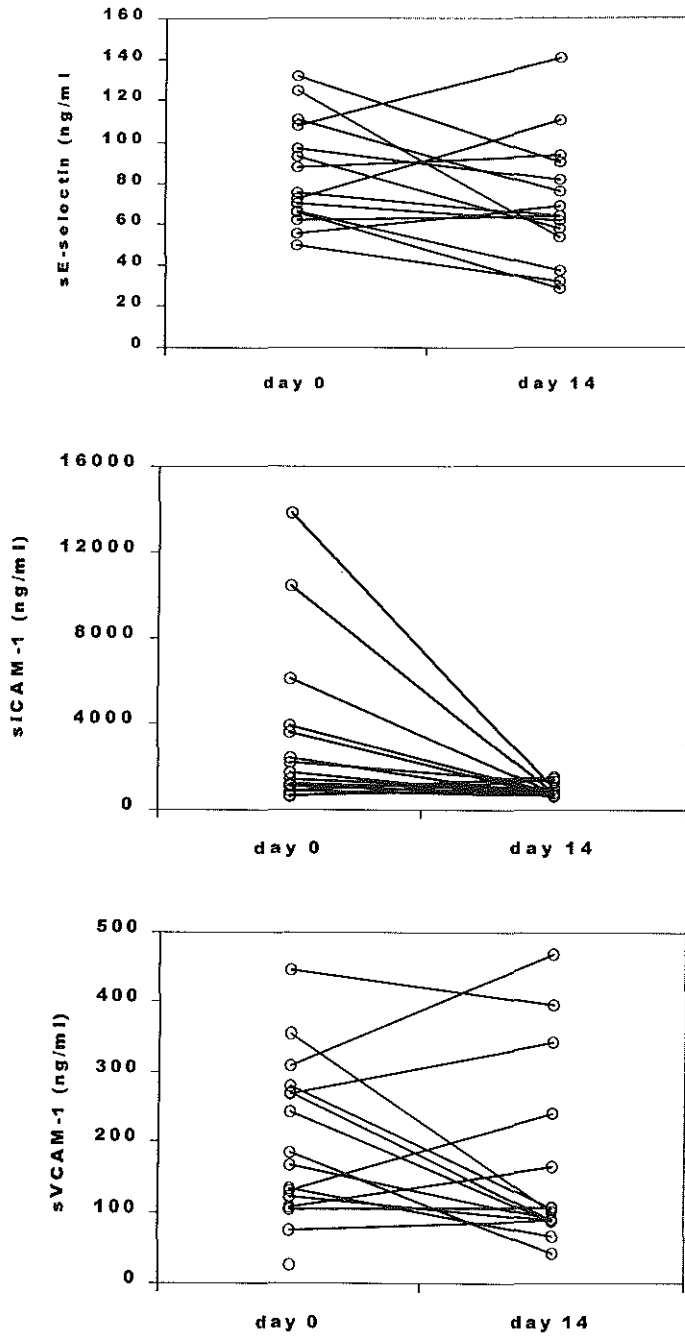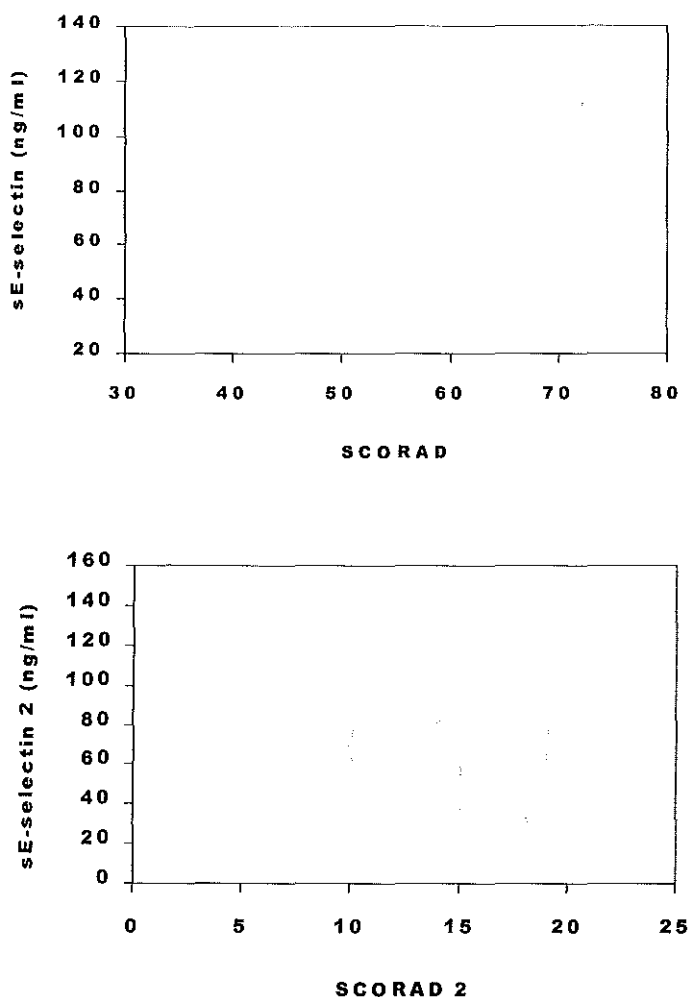
Figure 3.

A) Relationship between objective SCORAD and levels of sE-selectin at the beginning of the treatment (Rank Spearman's correlation: r=0.54, p<0.05). B) Relationship between objective SCORAD and levels of sE-selectin after 2 weeks of treatment (Rank Spearman's correlation: r=-0.52, p<0.05).
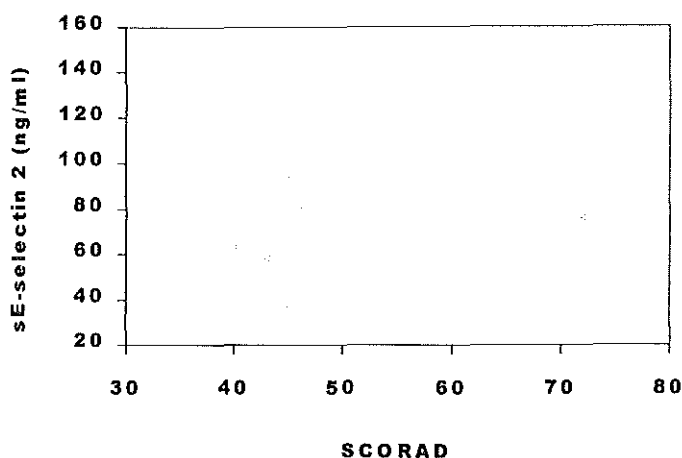
Figure 4.

Relationship between objective SCORAD at the beginning and levels of sE-selectin after 2 weeks of treatment (Rank Spearman' correlation: r=0.53, p<0.05)

Most importantly, the level of sE-selectin after 2 weeks of treatment still correlated significantly with the SCORAD at the beginning (r=0.53; p<0.05) *(Figure 4)*. Thus, while the severity of AD had dramatically decreased, the initial severity of disease was still reflected by the level of sE-selectin after 2 weeks. Furthermore, we noticed that children with high levels

of sE-selectin at the beginning tended to have high levels of sE-selectin after treatment as well (r=0.45; p=0.09) *(Figure 5)*.

Six children had asthma diagnosed by a physician of whom three children had current asthma medication with inhalation corticosteroids, but none of the children had current wheezing. There was no significant difference in the levels of sE-selectin, sICAM-1 and sVCAM-1 between the children with and without a diagnosis of asthma (p>0.05).

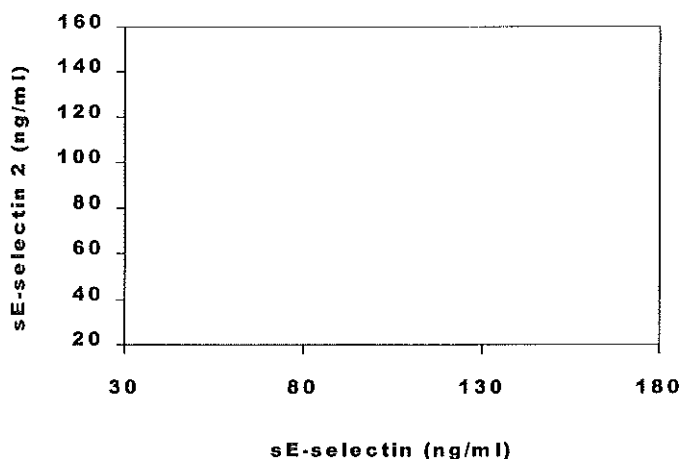Figure 5.

Relationship between the levels of sE-selectin at the beginning and after 2 weeks of treatment (Rank Spearman's correlation: r=0.45, p=0.09).

Discussion

The results of the present study indicate that sE-selectin is a marker of long-term activity of atopic dermatitis (AD), whereas sICAM-1 reflects short-term changes in the activity of AD. In contrast, sVCAM-1 was not related with the severity of AD. In agreement with the results of previous studies from our group[16,17] and others[7,11-13,27] we observed a clear correlation between the objective SCORAD and the levels of sE-selectin at the beginning of the treatment. We hypothesized that the treatment, which caused a dramatic improvement in the symptoms of up to 90%, would result in a profound decrease in the levels of sE-selectin. However, we failed to notice a significant decrease in the levels of sE-selectin. This was unexpected because the change in symptom score caused by the treatment was much higher than the variation in the symptom score at the beginning.

Table II

Longitudinal studies on the change in the levels of
sE-selectin by treatment of AD

Intervention	Period between measurements of sE-selectin	Change in level of sE-selectin
Cyclosporin A[11] 4-4.5mg/kg	16 weeks	P<0.0001
Topical corticosteroids[12]	2-5 weeks	P<0.05
Topical corticosteroids[13]	4 weeks	P<0.01
Systemic corticosteroids[18]	4 days	Not significant
Not specified[27]	Not specified	P<0.01

Most importantly, we observed that the levels of sE-selectin after 2 weeks of successful treatment still correlated with the objective SCORAD at the start of the treatment. Taken together, these results indicated that the serum levels of sE-selectin reflect the severity of AD during periods of longer than 2 weeks and are not influenced by acute fluctuations in the severity of disease. According to this finding, the seemingly conflicting results from previous longitudinal studies on sE-selectin can be explained *(Table II)*. Kagi et al,[11] Czech et al,[12] and Furue et al[13] reported a significant decrease of sE-selectin levels after 16 weeks, 2-5 weeks, and 4 weeks of treatment, respectively. In contrast, there was no significant decrease in sE-selectin after 2 weeks of treatment in our study or 4 days of treatment in the study by Halmerbauer et al.[18] Noteworthy, conventional treatment with topical steroids significantly decreased sE-selectin levels if measured after more than 2 weeks,[11-13] whereas even highly effective therapies associated with a major improvement in AD (systemic corticosteroids,[18] potent corticosteroids under occlusion), did not significantly decrease sE-selectin levels during the first 2 weeks of treatment.

Thus we propose sE-selectin as a marker of long-term activity of AD and demonstrate that the levels of sE-selectin after 2 weeks of successful treatment still reflect the original symptom score, despite major clinical improvement. We can only speculate on the underlying mechanisms of this finding, since little is known on the mechanisms of clearance and half-life of soluble adhesion molecules. Kinetics of the expression of soluble adhesion molecules may well be of relevance for detection as a marker of disease. The rates of release, binding and clearance reflected by blood concentration-time curves will partly determine the applicability of such levels in reflecting a localized disease process. After stimulation of endothelial cells with IL-1 and TNF-α in vitro, the levels of sE-selectin reached a maximum after 6 to 12 hours and dropped below detection limit after 24 hours.[15] In contrast, sICAM-1 gradually increased over a period of 24 hours and remained stable for 3 days. Thus, the production and release rate of sE-selectin does not explain our finding of sE-selectin as a marker of long-term activity of AD. However, differences in binding and clearance between sICAM-1 and sE-selectin may explain our observations.

In agreement with previous studies,[7,8] we noted a significant decrease in the levels of sICAM-1 after treatment, whereas we failed to show any correlation between the severity of AD before treatment and the levels of sICAM-1 before treatment. We do not know why the severity of disease at the beginning was only reflected by the levels of sE-selectin, because sVCAM-1 and sICAM-1 expression is also increased in lesional skin. One possible explanation is that ICAM-1 and VCAM-1 are constitutively expressed in normal skin and healthy appearing skin in patients with AD, whereas sE-selectin was only found in lesional skin.[14] Furthermore, sE-selectin expression is restricted to endothelial cells, whereas sICAM-1 and sVCAM-1 are expressed on other cell types as well. This indicates a higher specificity of E-selectin for affected skin and may explain why soluble E-selectin is a better indicator of disease severity in AD. However, E-selectin is not specific for AD and increased expression has been reported in psoriasis as well.[12]

Adhesion molecules have a crucial role in the migration of leukocytes. Membrane-bound adhesion molecules are glycoproteins, which mediate the attachment of leukocytes to endothelial cells thereby controlling migration. E-selectin and P-selectin mediate the first attachment of the leukocytes to the endothelial layer resulting in rolling along the endothelium. This weak binding, however, promotes stronger interactions between ICAM-1 and VCAM-1

on the endothelium and their leukocyte surface ligands; lymphocyte function associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4), respectively.[28]

E-selectin seems to have a critical role in allergic inflammation as it promotes the selective migration of both CD4+ and CD8+ T cells expressing the cutaneous lymphocyte antigen (CLA), which is a skin homing factor for CD45+ RO+ memory-type T cells.[29,30]

Adhesion molecules are rapidly upregulated on both endothelial cells and leukocytes upon exposure to cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4).[28] Increased expression of ICAM-1, VCAM-1 and E-selectin have been demonstrated in skin biopsies from chronic and acute AD lesions.[14,31] Six hours after intra-dermal allergen injection to sensitised individuals, enhanced expression of ICAM-1 and E-selectin was found in affected skin.[32] The soluble form of the adhesion molecules, sE-selec-tin, sP-selectin, sICAM-1 and sVCAM-1 originates from membrane bound molecules due to shedding or proteolytic cleavage.[33]

Little is known on the function of soluble adhesion molecules. It has been suggested that soluble adhesion molecules may have physiological implications, such as inhibition of adhesion by competitive binding. In contrast, it was also suggested that they may act in a proinflammatory manner by chemoattraction and by activating leukocytes.[34]

In conclusion, we advocate to use both a clinically based scoring system, such as the objective SCORAD and soluble E-selectin as an objective marker of the long-term activity of AD in studies on the pathogenic mechanisms of AD. Furthermore, the kinetics of soluble E-selectin has to be considered in the interpretation of the results.

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Cytokine production by peripheral blood cells from children with atopic dermatitis

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This manuscript is dedicated to the memory of Astrid Vredendaal

Abstract

Assessment of the capacity of immunocompetent cells to produce IL-4 and IFN-γ requires a multistep procedure consisting of cell isolation (PBMC), stimulation culture and analysis of cytokine levels in supernatants. The DynaMIX DIA kit uses whole blood and combines cell stimulation and cytokine trapping by catching antibodies in a simple one-well format. In the present study IL-4 and IFN-γ production were investigated in both stimulated peripheral blood mononuclear cells (by Medgenix Screening Line ELISA) and in whole blood cell cultures (by DynaMIX DIA kit) in 12 children with atopic dermatitis (AD). Optimal culture conditions were established and the IL-4 and IFN-γ production were correlated to the severity of AD and the levels of soluble E-selectin and ICAM-1. No significant correlation was found in the cytokine production between the assessment using Screening Line ELISA and DynaMIX DIA kit.
IL-4 production by PBMC was correlated to the severity of AD (Rank Spearman's correlation: $r=0.93$; $p<0.0005$) while IFN-γ production was not. IL-4 and IFN-γ production in whole blood were not correlated to the severity of AD, which may be due to suboptimal culture conditions. Our data show that stimulation with Ca-ionophore and PMA in Yssel's

medium resulted in production levels of IL-4 and IFN-γ which were higher than stimulation with PHA in RPMI, while retaining a similar sensitivity and specificity compared to stimulation of PBMC using the Medgenix Screening Line ELISA.

Soluble E-selectin was correlated to the severity of disease (r=0.96; p<0.005) and to the IL-4 production in PBMC (r=0.99; p<0.05) .

We conclude that when incorporating the culture and stimulation conditions we present here (Ca-ionophore and PMA in Yssel's medium) the DynaMIX DIA kit can be used effectively for cytokine analysis in children suffering from AD.

Introduction

Atopic diseases are generally considered to be the resultant of an aberrant cytokine synthesis in genetically predisposed individuals in response to exposure to common allergens. Importantly, the Th1-Th2 paradigm has been used to explain this cytokine dysbalance [1, 2] as a direct consequence of an excessive number and/or activation state of Th2 type cells, mainly characterized by the predominant production of IL-4, IL-5, IL-10 and IL-13 [3, 4, 5]. Th1 type cells, producing IFN-γ, are suggested to restore this balance due to crossregulation on the Th2 activity [6]. IL-4- and IL-13-dependent serum IgE levels are increased in 80 to 85% of all patients with AD [7, 8] with the highest concentrations when allergic asthma and rhinitis coexist [9]. The skin lesions of AD are characterized by a chronic inflammation involving mast cells, eosinophils and T lymphocytes. The accumulation of these inflammatory cells is a consequence of the upregulation of endothelial adhesion molecules [10]. Increased expression of endothelial adhesion molecules, like intercellular adhesion molecule-1 (ICAM-1) and E-selectin have been demonstrated in skin biopsies from chronic and acute AD lesions [11, 12]. As a result of shedding or proteolytic cleavage soluble adhesion molecules are present in the circulation. We have found a positive correlation between the serum levels of soluble E-selectin and the severity of AD in children [13, 14]. The severity of AD can be measured by using the SCORing Atopic Dermatitis (SCORAD) system, which reflects the consensus of the European Task Force on Atopic Dermatitis [15-17]. Various studies have tried to correlate the severity of AD to the activity of Th2 type

cells or Th1 type cells by measuring IL-4, IFN-γ or IL-13 mRNA expression and protein production levels by peripheral blood mononuclear cells (PBMC) [18, 19, 20]. Generally such a simple correlation does not exist, probably due to the very low production rate and low precursor frequency of IL-4 (or IL-13) producing cells. Furthermore the Th1/Th2 paradigm has been questioned [21, 22] and Th1 type cells and related cytokines too have been shown to be important in atopic dermatitis. In lesional skin of atopic dermatitis increased expression of IFN-γ has been demonstrated [23-25]. Several studies support a biphasic model with IL-4 being responsible for the initiation of allergic skin reactions whereas IL-5 and IFN-γ predominate in chronic AD lesions and in late phase allergic skin reactions [23-26].

In order to further investigate the Th1/Th2 balance in children suffering from AD, we analysed the production of IL-4 and IFN-γ in stimulated peripheral blood mononuclear cells and compared this with whole blood cell cultures. Furthermore optimal culture conditions were established and the IL-4 and IFN-γ production was correlated to the severity of AD, the levels of total IgE, and the levels of soluble E-selectin and ICAM-1.

Materials and methods

Patients

Twelve children (age 15 to 32 months) with AD according to the criteria of Hanifin and Raijka [27] were included. After informed consent peripheral blood was obtained. Severity of AD was severe (SCORAD>40) in 1 child, moderate (SCORAD 15-40) in 2 and mild (SCORAD<15) in 9 children according to the objective SCORAD [16]. Eight children used topical corticosteroids and / or systemic antihistamines as treatment of AD. None of the children had asthma or any other chronic disease. The local Ethical Committee approved this study.

Clinical scoring system

The severity of disease was scored using the objective SCORAD [16, 17] which includes the assessment of two items in a standardised manner: a) extent (applying the rule of nine) and

b) intensity (erythema, edema/papulation, oozing/crust, excoriation, lichenification and dryness on a scale from 0 to 3). This scoring system reflects the consensus of the European Task Force on Atopic Dermatitis [15].

Determination of soluble adhesion molecules

sE-selectin and sICAM-1 were measured in plasma by ELISA as described previously [13]. Briefly, 96-wells microtitre plates were coated with monoclonal antibodies to sICAM-1 (HM.2, 3 µg/ml) or sE-selectin (ENA1, 10 µg/ml) in phosphate-buffered saline (PBS) followed by saturation with 1% bovine serum albumin. Standard or diluted samples were added in 100 µl/well. After two hours at room temperature, the plates were washed and incubated for one hour with biotin labelled anti-ICAM-1 or anti-E-selectin monoclonal antibodies. All antibodies were a kind gift of dr. W.A. Buurman (University of Maastricht, The Netherlands). After washing streptavidin-conjugated peroxidase was added. Peroxidase activity was determined by addition of 2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS). The reaction was stopped with H_2SO_4 and read at 414 nm. The concentration of sICAM-1 and sE-selectin were determined by interpolation from the standard curve. The detection limits of these ELISA assays were 4.0 pg/ml for sICAM-1 and 2.0 pg/ml for sE-selectin. sVCAM-1 was measured in plasma by a similar procedure using a commercially available test kit (R&D Systems Europe, Abbigdon, UK)

Total IgE was determined by CAP system (Pharmacia Upjohn, Uppsala, Sweden).

Culture conditions

Whole blood cells were cultured using the DynaMIX DIA kit according to the manufacturer's instructions (Medgenix Diagnostics, Fleurus, Belgium). Briefly, 25 µl of whole blood was diluted in 200 µl of RPMI medium supplemented with phytohemagglutinine (PHA, 10 µg/ml) and incubated for 24 hours (both for IL-4 and IFNγ) at 37°C. Alternatively, peripheral blood mononuclear cells (PBMC) were isolated from heparinised venous blood by density gradient centrifugation on Ficol-Hypaque (Pharmacia) [3].

Staining whole blood and isolated PBMC with anti-CD3 (Leu4) monoclonal antibodies and subsequent FACscan analysis permitted determination of the relative amount of T cells.

Equal concentrations of T cells were cultured for 24 hours by stimulation with Ca-ionophore (1 µg/ml; a23187, Sigma, St. Louis MO, USA) and phorbol-12-myristate-13 acetate (PMA, 2ng/ml; Sigma) in Yssel's medium (YM) [3,4]. Moreover, reciprocal stimulation conditions were also investigated by using Ca-ionophore and PMA in Yssel's medium in the DynaMIX DIA kit and by stimulating PBMC with RPMI supplemented with PHA. Furthermore, the cytokine production of PBMC with or without stimulation by PHA was analysed in cultures with RPMI-1640 (used in our laboratory), RPMI supplied in the DynaMIX DIA kit, or Yssel's medium.

Assay for IL-4 and IFN-γ

In the conventional ELISA assay levels of IL-4 and IFN-γ were determined in the supernatants by Screening Line ELISA kits (Medgenix Diagnostics SA) according to the manufacturer's instructions. The lower detection limit was 30 pg/ml for IFN-γ and 3 pg/ml for IL-4.

In the DynaMIX DIA kit IL-4 and IFN-γ are captured on the surface of microtiter plates that are coated with specific monoclonal antibodies directed against the cytokine [28, 29]. Subsequent to the removal of the supernatant, the captured cytokine can be detected by a specific anti-cytokine peroxidase-labeled monoclonal antibody and tetramethylbenzidine (TMB), quantified by a spectrophotometer at 450 and 490 nm. The lower detection limit was 2 pg/ml for IL-4 and 1 pg/ml for IFN-γ.

Statistics

Statistical analyses were performed with SPSS 6.1. Non-parametric data were tested using the Mann-Whitney test. SCORAD, sE-selectin, sICAM-1, IL-4 and INF-γ production were correlated by using the Rank Spearman's correlation coefficient. In either test, P values less than 0.05 were considered significant.

Results

Culture conditions

Stimulation experiments were performed under several different culture conditions. Stimulation was performed on whole blood cells (WBC) diluted in RPMI medium supplemented with PHA and on PBMC diluted in Yssel's medium and supplemented with PMA and Ca-ionophore. Moreover, reciprocal stimulation conditions were also assayed by using Ca-ionophore and PMA in Yssel's medium in the DIA kit and by stimulating PBMC with RPMI supplemented with PHA.

Table 1   Cytokine production in stimulated whole blood cells (WBC) and PBMC

	WBC (DIA)	PBMC(DIA)	PBMC(ELISA)
		IFN-γ (pg/ml)	
RPMI	<1	<1	<1
RPMI+PHA	11±2.4	<1	<1
YM	<1	1.4±0.6	<1
YM+Ca/PMA	2.7±1.2	13±3.4	97.5±12.9
		IL-4 (pg/ml)	
RPMI	<2	<2	<2
RPMI+PHA	21.5±4.9	<2	<2
YM	16±3.8	<2	15.5±2.8
YM+Ca/PMA	90.5±14.1	50±12.3	95±16.7

Cultures were performed with whole blood cells (WBC) and isolated peripheral blood mononuclear cells (PBMC) cultured for 24 hours in RPMI-1640 and YM culture media. Stimulation was performed either with PHA or with Ca-ionophore plus PMA. DIA refers to stimulation and ELISA in DynaMIX kits, while ELISA refers to stimulation in culture plates and assaying of culture supernatants in separate ELISA plates. Representative results of one out of 4 experiments are shown. Cytokine production was measured in screening line ELISA and levels of IL-4 and IFN-γ are expressed in pg/ml (n=4; mean ± 1 SD).

The results (table 1) suggested that in both assay formats using WBC or PBMC stimulation with Ca-ionophore and PMA in combination with Yssels's medium were superior in the production of IL-4 and IFN-γ as compared with stimulation by PHA in RPMI. Stimulation of whole blood by Ca-ionophore and PMA in Yssel's medium revealed similar levels of cytokines as compared to stimulation of WBC in RPMI supplemented with PHA as provided in the DIA kit. Cultures of PBMC stimulated by PHA in RPMI appeared to be less viable when compared to cultures in Yssel's medium (data not shown).

The effect of different culture conditions on PHA-induced cytokine production of PBMC cultured for 24 hours is shown in table 2. These results demonstrated that stimulation by PHA induced higher production levels of IL-4 and IFN-γ when used in combination with Yssel's medium. The use of RPMI from either source did not result in detectable increases in cytokine production after stimulation.

Table 2 Influence of PHA stimulation and culture medium on cytokine production.

	IL-4	IFN-γ
RPMI$^{\#}$	67	81
RPMI$^{\#}$+PHA	91.4	100
RPMI*	<3	71
RPMI*+PHA	<3	85
YM	<3	17
YM+PHA	19.9	1242

All cultures were performed with isolated PBMC cultured for 24 hours. Representative results of one out of 4 experiments are shown. Cytokine production was measured in Screening Line ELISA and levels of IL-4 and IFN-γ are expressed in pg/ml (n=4; mean ±1SD). RPMI$^{\#}$ refers to RPMI-1640 while RPMI* refers to the RPMI supplied in the DynaMIX DIA kit. The source of PHA is identical and is used at 10µg/ml.

Comparison of assays

For comparison of the quantitative determinations of IL-4 and IFN-γ by DymaMIX DIA kit and by Screening Line ELISA the supplied cytokine standards were used. In the conventional ELISA assay two-fold dilutions of Yssel's medium starting at 500, 250, 125,

63.5, 31.25 and 16 pg/ml IFN-γ and the DynaMIX DIA kit standard of 160, 80, 40, 20 and 0 pg/ml IFN-γ from Medgenix were tested. The sensitivity of this ELISA for both IFN-γ standards was identical both in detection limit of the assay (1 pg/ml) and in fitting parameters of the standard curve according to the 4-parameter log/logit transformation (Figure 1, upper panel). Based on the two standard curves the specific activity of the IFN-γ standard was calculated to be 8.25 x 10^8 U/mg.

Similar experiments were performed for IL-4 using the Medgenix Screening Line ELISA standard. (Figure 1, lower panel). In the conventional ELISA assay two-fold dilutions of Yssel's medium starting at 1125, 500, 250, 125, 63.5, 31.25, 15.63 and 0 pg/ml of the of the IL-4 standard from Medgenix were tested. In this ELISA also the DynaMIX DIA kit IL-4 standard of 1125, 500, 425, 375, 90, 80, 60, 40 and 0 pg/ml was applied in Yssel's medium. The sensitivity of this ELISA for both IL-4 standards was identical both in detection limit of the assay (2 pg/ml) and in fitting parameters of the standard curve according to the 4-parameter log/logit transformation (Figure 1, lower panel).

The use of the DIA kit permits the storing at −20°C of the samples after stimulation and before further analysis. Direct analysis of freshly diluted standards for IL-4 and IFN-γ versus analysis of frozen samples revealed similar standard curves (Figure 2).

Cytokine analysis of blood from allergic children
Whole blood and purified PBMC obtained from children suffering from AD and healthy control children were analysed using the various culture conditions for the levels of IL-4 and IFN-γ production after stimulation (Figure 3). The cytokine production levels in unstimulated cultures were below detection level except in one patient who had high spontaneous production of both IL-4 and IFN-γ in unstimulated conditions. This was the only patient with severe and widespread AD and an objective SCORAD of 49. The IL-4 levels produced by PBMC stimulated by Ca-ionophore and PMA in YM in the DIA kit format correlated significantly with the total IgE levels in the sera of these children (Figure 4), while the IL-4 levels as determined by analysing whole blood in the DIA kit (stimulated with PHA in RPMI) did not correlate with the total IgE levels (data not shown).
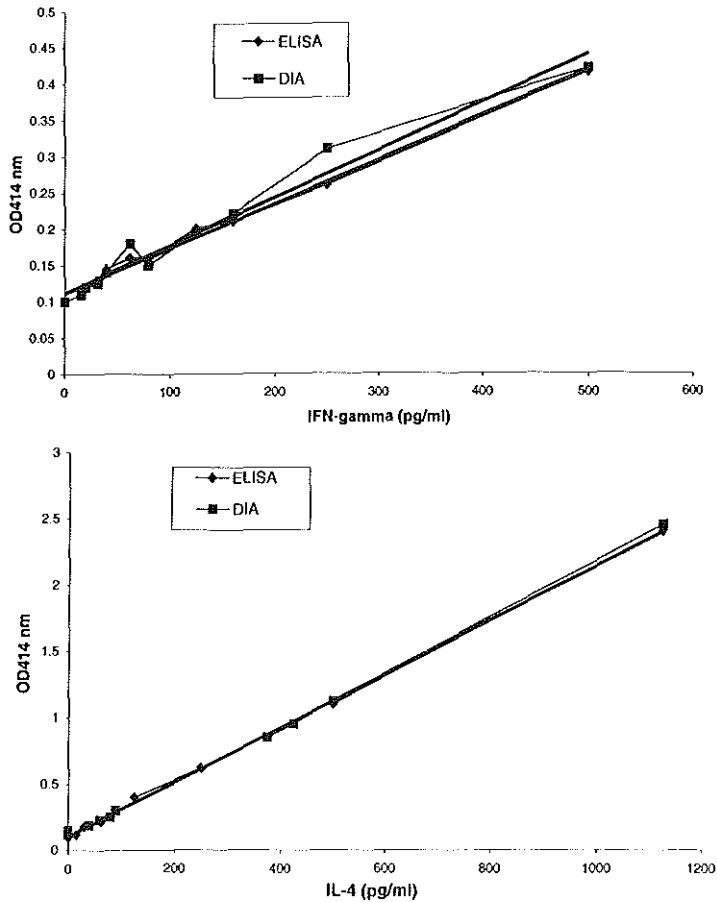
Figure 1

ELISA results performed on IL-4 (upper panel) and IFN-γ (lower panel) standards used in ELISA and DynaMIX DIA kits. Dilutions of available IL-4 and IFN-γ standard preparations present in the ELISA kit (Medgenix Screening Line) and in the DynaMIX IL-4 and IFN-γ DIA kit (Medgenix) were made in YM and tested in a routine ELISA resulting in OD_{414nm} readings after 90 min.
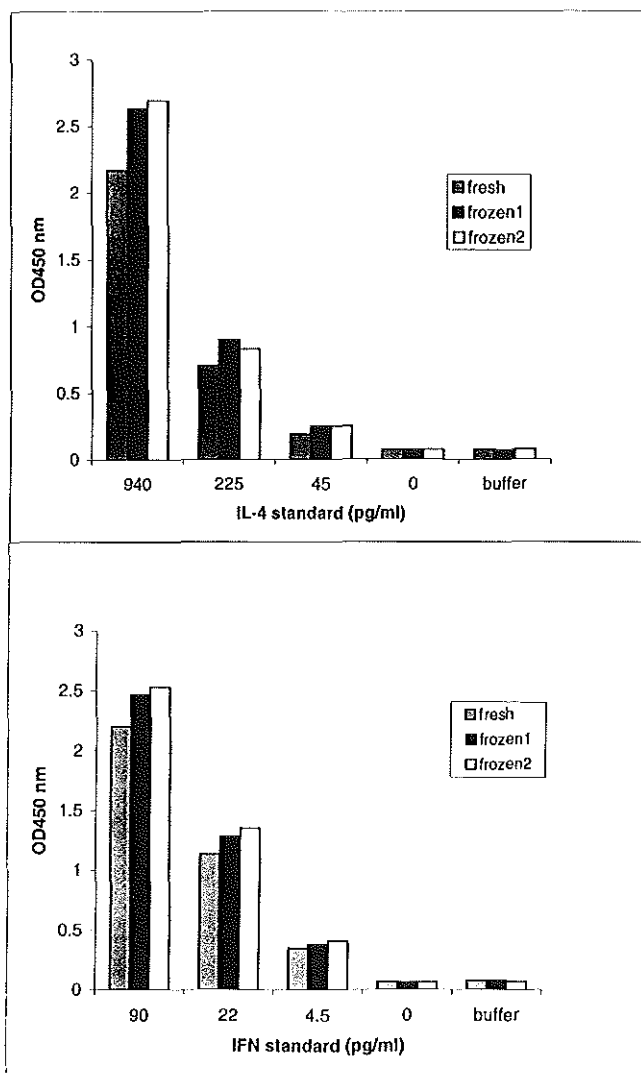
Figure 2

Recovery of activity of DynaMIX DIA kit standards of IL-4 (upper panel) and IFN-γ (lower panel) after frozen storage. Various dilutions of standards were freshly tested (fresh), or after freezing and immediate thawing (frozen 1), or freezing and subsequent storage of two weeks before thawing and testing (frozen 2). All samples were tested in ELSIA and results are presented as OD_{450nm} versus dilutions.
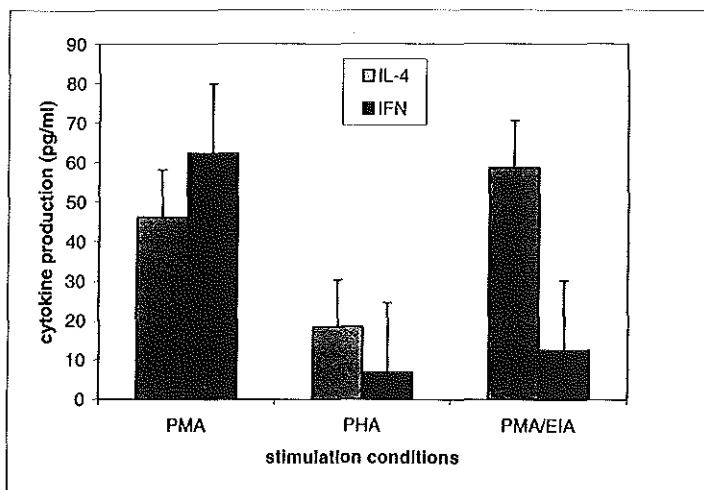
Figure 3

Cytokine production (IL-4 and IFN-γ) after polyclonal stimulation of isolated PBMC stimulated with Ca-ionophore and PMA in YM in DynaMIX DIA kit (PMA), stimulation of whole blood with PHA in RPMI in the DynaMIX DIA kit (PHA), or stimulation of PBMC with Ca-ionophore and PMA and analysing the culture supernatant by regular ELISA (PMA/ELISA). Blood was obtained from all 12 allergic children suffering from AD that were included in this study. The results are expressed in ng/ml and represent the mean and SD of 4 independent experiments.

When comparing the cytokine production between the Screening Line ELISA and the DynaMIX DIA kit no significant correlation was found (IL-4: r=0.046 p=0.8; IFN-γ: r=0.34 p=0.3) (data not shown).

IL-4 production by PBMC (Screening Line ELISA) was correlated to the severity of AD (Rank Spearman's correlation: r=0.93; p<0.05) (Figure 5), while IFN-γ production was not (data not shown). IL-4 and IFN-γ production in whole blood (DynaMIX DIA kit) were not correlated to the objective SCORAD (IL-4: r=0.41; IFN-γ: r=0.81, p=0.55; Figure 6).
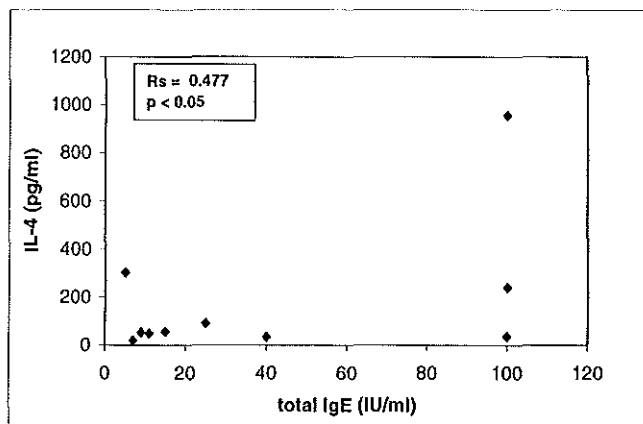
Figure 4

Relationship between total IgE levels in the serum (in IU/ml) of the allergic children included in this study (n=12) and the IL-4 production. IL-4 production in PBMC stimulated with Ca-ionophore and PMA in YM using the DynaMIX DIA kit and expressed in pg/ml.
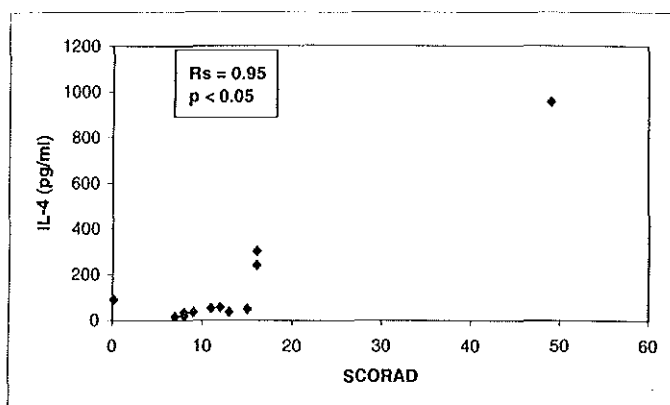

Figure 5

Relation between IL-4 production and severity of AD as measured by SCORAD. IL-4 production in PBMC stimulated with Ca-ionophore and PMA in YM using the DynaMIX DIA kit. The figure shows the relation between IL-4 production and objective SCORAD in 12 children with AD (Rank Spearman's correlation: r=0.95; p<0.05).
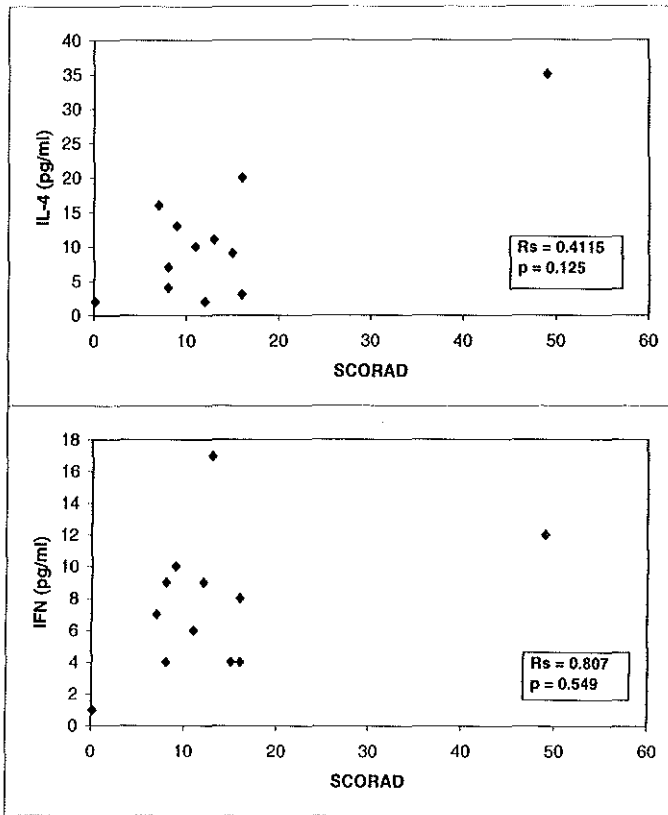
Figure 6

Relation between IL-4 production (upper panel) and IFN-y production (lower panel) with the severity of AD as measured by SCORAD. IL-4 and IFN-y production in PBMC stimulated with Ca-ionophore and PMA in YM using the DynaMIX DIA kit. The figure shows the relation between cytokine production and objective SCORAD in 12 children with AD.

Levels of sE-selectin and sICAM-1

Figure 7 shows the relation between the levels of sE-selectin and the severity of AD (Rank Spearman'correlation; r=0.96; p<0.05). The levels of sICAM-1 were not significantly correlated to the objective SCORAD. Furthermore we found a significant correlation between levels of sE-selectin and IL-4 production in stimulated PBMC (data not shown). Even when the patient with the high SCORAD value (49) was omitted from the analysis, the correlation between IL-4 and sE-selectin was still significant (p<0.05).
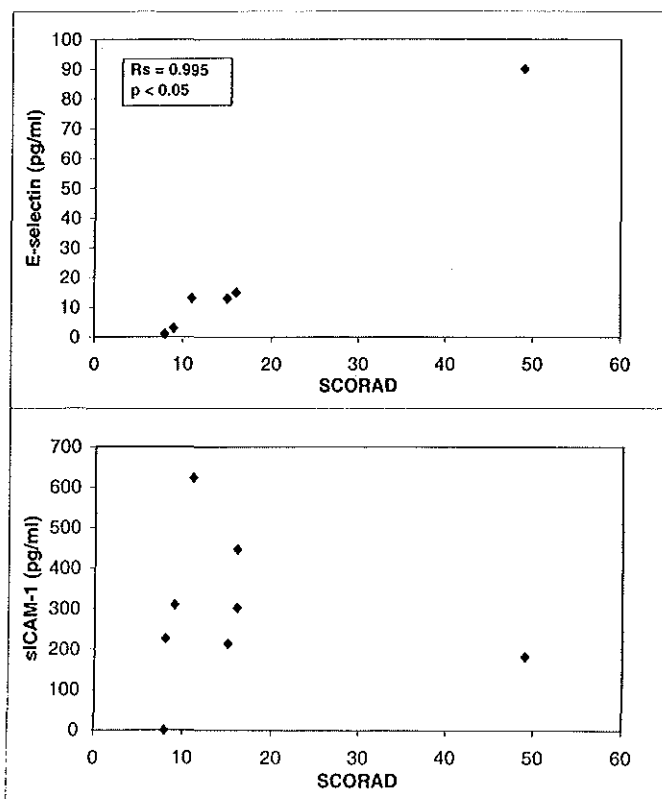
Figure 7

Relation between serum levels of sE-selectin (upper panel) and sICAM-1 (lower panel) (in pg/ml) and the severity of AD as measured by SCORAD (n=8).

Discussion

In the present study we found a significant positive correlation between the severity of AD (SCORAD) and the IL-4 production of stimulated PBMC. The dominance of Th2-like cells in the affected skin tissue of patients with AD was also demonstrated. Thus, these Th2 cells appear to be essential for the development of AD. Several studies have demonstrated

increased IL-4 and decreased IFN-γ mRNA expression and protein production in AD as compared to non-atopic controls. The most pronounced changes in cytokine pattern are found in severe AD while in patients with mild AD normal [19,20] or minimally deviating [5] cytokine patterns were found. This points to a relation between the severity of disease and the underlying immune devation. However, a correlation with the severity of AD using a validated clinical scoring system has rarely been established yet [5,22]. Contrary to asthma and allergic rhinitis, the extent and intensity of the inflammation can be assessed by a scoring system in AD. For this purpose we used the objective SCORAD which is based on a broad consensus of dermatologists.

As IL-4 and IFN-γ are usually undetectable in serum, the secretion ability of immunocompetent cells after stimulation is analysed. For this purpose usually a multistep procedure is applied consisting of cell isolation, stimulation, culture and analysis of cytokine levels in supernatants. The DynaMIX DIA kit combines cell stimulation and cytokine trapping by catching antibodies in a simple one-well format (28). A reliable quantification method can thus be employed without the need of an isolation procedure that may lead to uncontrolled cell activation and deprive cells of important interactions present in whole blood (29). While we found a significant correlation between the IL-4 production in PBMC (by Screening Line ELISA) and the objective SCORAD, there was no correlation between the IL-4 production in whole blood (by DynaMIX DIA kit) and the objective SCORAD. We consider this a consequence of suboptimal culture conditions used in the DynaMIX DIA kit.

Both IL-4 and IFN-γ production in unstimulated blood was below detection limit except in the patient with the highest SCORAD. This was the only patient with severe AD. Interestingly, both IL-4 and IFN-γ production were increased suggesting also a role for IFN-γ in severe AD. This finding is in agreement with an earlier report of spontaneous IL-4 production in unstimulated cultures of PBMC from patients with high IgE levels and severe atopic dermatitis [30]. In other studies spontaneous production of intracellular IFN-γ has been reported in AD [31,32]. It can be assumed that in those patients with severe AD previous in vivo activation, presumably of T cells, has occurred.

The present study shows a significant correlation between sE-selectin and the severity of disease, which is in line with earlier reports [13, 14, 33-35]. E-selectin seems to have a critical role in allergic inflammation as it promotes the selective migration of both CD4+ and

CD8+ T cells expressing the cutaneous lymphocyte antigen (CLA) which is a skin homing factor for CD45+ RO+ memory-type T cells [36, 37]. Furthermore we demonstrate a highly significant correlation between the levels of sE-selectin and the production of IL-4 in stimulated PBMC of children with AD. This has not been reported previously, as far as we are aware. Although the expression of adhesion molecules is controlled by locally produced cytokines, it is unlikely that there is a direct causal relation between the increased IL-4 production and the increased sE-selectin levels. IL-4 has been shown to inhibit the induction of E-selectin expression by both IL-1 and tumor necrosis factor α (TNF-α) [38]. Therefore other cytokines than IL4 that are increased in atopic dermatitis must be responsible for the increased expression of E-selectin. Candidates are IL-1[39, 40], TNF-α [38, 39, 40] and IL-3 [41].

When using polyclonal stimulation by Ca-ionophore and PMA, all different culture media employed will facilitate cytokine production equally well. However, mitogenic stimuli, like PHA, stimulate most, but not all T-cell subsets, in an antigen-presenting cell-dependent fashion. Optimal stimulation conditions are then dependent on the presence of multiple cellular subsets and are therefore best arranged by testing whole blood samples, mimicking the in vivo situation. These stimulation conditions ensure the detection of possible intrinsic differences among the T cells in the various patient groups that would reveal changes due to environmental stimuli or the expression of the atopic disease. Only when such intrinsic differences are not apparent among the T cell subsets it is useful to interpret differences under allergen-specific stimulation conditions. As in all experimental test systems identical antibody populations were used, and the tests were all performed in microtiterplates, and therefore the specificity of the assays is identical. All ELISA procedures display similar sensitivities enabling the detection of cytokine levels after polyclonal stimulation of PBMC in vitro.

Our data show that when using different culture conditions than advised by the manufacturer (Yssel's medium with Ca/TPA instead of RPMI with PHA) the sensitivity and specifity of the DynaMIX DIA kit were rendered comparable to the ones in stimulated PBMC using the Medgenix Screening Line ELISA. The very fact that the DynaMIX DIA kit permits storage of the samples after stimulation before further analysis increases its practical use. The comparison between freshly diluted standards and frozen ones revealed similar standard

curves. Moreover, since both the stimulation and the assay are performed in the same plate makes the system well suited for a high sample throughput as compared to the conventional way of analysis. Furthermore, the omission of of cell separation presents further time saving. When incorporating the culture and stimulation conditions we present here (Ca-ionophore and PMA in Yssel's medium) this system presents similar specificity, sensitivity and secretion levels as compared to our conventional analysis. Based on its better practical performance the presented DynaMIX DIA kit can be used effectively in cytokine analysis in clinical immunology. However, though both systems perform rather similar, it is advised not to intermingle the use of both systems.

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Chapter 5

Therapy of atopic dermatitis

5.1 Advances in the treatment of atopic dermatitis with special regard to children
Curr Probl Dermatol 1999; 28: 56-63.

5.2 Fluticasone propionate 0.05% cream once daily versus Clobetasone butyrate 0.05% cream twice daily in children with atopic dermatitis
J Am Acad Dermatol 1998; 39(2 Pt 1): 226-31.

5.3 Treatment of erythrodermic atopic dermatitis with Wet-Wrap Fluticasone propionate 0.05% cream : emollient 1:1 dressings
J Dermatol Treatment 1999; 10: 73-74.

5.4 Efficacy and safety of Wet-Wrap dressings in children with severe atopic dermatitis: influence of corticosteroid dilution and duration of treatment
Submitted

Advances in the treatment of atopic dermatitis with special regard to children

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Introduction

Current therapy of atopic dermatitis (AD) is directed at suppressing the inflammation and reducing triggering factors. The goals of current standard therapy are improvement of the skin-barrier, blocking of receptors and reduction of the inflammatory infiltrate [1, 2]. Advanced treatment of severe AD in children includes: (1) general aspects; (2) preventive measures ; (3) an individualized therapeutic regimen and use of emollients; (4) topical application of corticosteroids; (5) topical or systemic antibiotics and topical antiseptics; (6) elimination diets; (7) systemic treatment in recalcitrant disease (cyclosporin); (8) other therapies, and (9) experimental therapy.

It is important to score the severity of AD to evaluate the effects of therapy. Different systems are available for scoring the severity of AD. The European Task Force on Atopic Dermatitis developed a scoring system based on consensus [3]. This system was called the SCORAD-index and contained objective and subjective criteria. Subjective criteria are difficult to evaluate, especially in young children. Therefore we use the objective SCORAD. This scoring-system is an excellent instrument for use in trials and studies. Because the SCORAD is time-consuming, for routine use we devised a simpler scoring system from this and called it TIS: three-items severity score, involving the representative erythema, oedema and excoriations [Wolkerstorfer et al., submitted]. Each item can be graded on a scale from 0-3. Maximum TIS-score is 9.

In a validation retrospective study we determined the TIS-score of 152 children [van Meurs, internal report]. Objective SCORAD scores of all these children were already known. The

correlation between the TIS scores and the SCORAD scores was calculated. This correlation was very good. (0.8746 p = 0.001). In a prospective study we obtained the same results [Wolkerstorfer et al., submitted]. The TIS-score is a good assessment method for routine use. This may be a satisfactory solution to the problem of assessing the severity of AD, especially in the general practice.

General aspects of current therapy

After daily bathing (for a maximum of 10 min), the use of emollients and soap substitutes are recommended. Prevention of triggering factors is important and will be dealt with in more detail later. The mainstays of topical treatment of AD are the use of emollients and corticosteroids, although adjunctive measures are important. Tars have been used for many years in the treatment of AD [4]. Tars are antipruritic and antiproliferative and efffective in subacute and chronic AD. Major drawbacks of tar are that it is messy, odorous, phototoxic and may induce folliculitis. A lot of concern has arisen due to possible carcinogenicity, but the incidence of skin cancer in tar-treated patients is very low [5]. We still often use coal tar at low concentrations, for example solutio carbonis detergens 10% in a zinc oxide-containing ointment.

Day care with education for the parents and the children and psychosocial support will improve the care of children and parents. Minor behavioral problems and distress in the parents are important features in severe AD in early childhood [6]. Children and their families need education and the support of health care professionals [7]. Long hospitalization is stressful for the well-being of the children, is extremely expensive for society, and should be limited as much as possible.

Preventive measures

One should avoid irritants such as detergents and wool. Early exposure to allergens may result into sensibilisation. Therefore preventive sanitization procedures (for house dust mite, animals and other allergens) should be undertaken from early infancy onwards. Emotional stress should be avoided [7]. Psychological factors such as frustration and anxiety will complicate AD and trigger worsening of the disease.

Interventions to prevent the switch from AD to asthma are difficult to evaluate. Two candidates to prevent the switch are cetirizine and loratidine. Studies are currently in progress to evaluate these. The Early Treatment of Atopic Child trial concerns a unique long-term study in 817 children with atopic dermatitis [AD] to determine the risk of developing asthma as well as the preventive efficacy of cetirizine. During the three years-study, a part of those children is likely to develop asthma and it will be of special interest to assess whether this can be predicted by clinical or laboratory variables. Blood and urine samples were taken at several visits and stored for additional research to study the allergic march from AD to asthma. Several markers were investigated to evaluate the inflammation in order to obtain a more detailed insight into the process of allergic inflammation in the skin or the lungs. The first results of this trial have only recently become available and have just been published. There are indications that cetirize reduces the chances for developing asthma in some subgroups of young children with AD [8]. In a double-blind placebo-controlled study, cetirizine halved the number of patients developing asthma in the subgroups sensitised to grass pollen or house dust mite, that included 20% of the study population [8]. Asthma preventing capacities are also claimed for loratadine, but a study is still in progress on.

Corticosteroids

Topical corticosteroids are the primary mainstay of the topical treatment of childhood AD [4]. Depending upon the age mild to moderate potent corticosteroids are used in children. Most dermatologists prefer in children to use hydrocortisone acetate and triamcinolone acetonide in children. Stronger preparations usually carry higher risks of systemic side-effects.

New preparations such as fluticasone propionate, mometasone fuorate methylprednisolone aceponate and prednicarbate are interesting because of their effectivity and increased benefit-risk ratios. Mometasone furoate 0.1% is already registered in the USA for pediatric age. Local and systemic side effects are negligible except for slight skin thinning and hair growth (own observations) [9,10]. Incidentally striae were observed in (pre)puberal children (own observations). These formulas are suitable for application with the wet wrap technique because of their increased benefit-to- risk ratio.

Adrenal suppression is a potential complication of topical corticosteroid treatment in AD [11]. Fasting cortisol levels, body weight, urinary excretion of cortisol and ACTH test are used to check adrenal suppression. The ACTH test is the most reliable test, but is patient unfriendly and stressful. Patel et al [11] studied 14 prepubertal children with moderate to severe AD using the ACTH test to detect subtle changes in adrenal glucocorticoid function. All children used hydrocortisone acetate ointment and moderately potent preparations intermittently. No suppression of adrenal glucocorticoid function was observed [11]. In a comparative study by Vernon et al. [10] of 0.1% mometasone furoate cream and 1.0% hydrocortisone cream in 48 children with AD, only 1 child showed transiently suppressed cortisol levels. This child was treated with 1.0% hydrocortisone acetate cream. Wolkerstorfer et al. [12] studied 21 children who were treated double-blind with either 0.05% fluticasone propionate or 0.05% clobetasone butyrate. Once daily application of 0.05% fluticasone propionate was equal to twice daily application of 0.05%) clobetasone butyrate. No influence on the levels of excreted cortisol in the urine was observed [12].

Wet Wrap Technique

In 1991 Goodyear et al. [13] described the wet wrap technique for the treatment of AD. Wet wrap treatment plays an important role in the management of childhood AD which does not respond to first-line treatment. Wet wrap treatment is also effective in adult erythrodermic AD. Different variations of this technique may be used (bandages soaked with emollients or diluted corticosteroids. or bandages soaked with warm water or re-wetting or not re-wetting the bandages every hour). However, controlled studies are lacking.

The wet wrap technique is a kind of occlusive treatment. The absorption of the topical corticosteroid is increased under occlusion, while hydration of the skin leads to a 4- to 5-fold increase in absorption [4]. Wet tubular dressings are used over emollients and diluted corticosteroids. Although rapid improvement in AD occurred, adrenal pituitary suppression limited its long-term use [13]. Betamethasone and even 1.0% hydrocortisone acetate application resulted in HPA-axis suppression. Fluticasone propionate (0.05%) is a corticosteroid which seems to have an improved benefit-to-risk ratio. Therefore it appears

to be suitable for the wet wrap technique for long-term treatment. This therapy with a potent diluted corticosteroid cream with minor systemic side effects is an interesting and promising option not only in children. but also in adults. The concentration of the 0.05% fluticasone propionate cream-emollient 1:1 dilution may be lowered to 10% diluted steroid cream. This lower concentration will further improve the safety of the treatment [Wolkerstorfer et al., in preparation].

Antihistamines

Oral antihistamines are often included in the therapy for AD. However, the exact role in the treatment is conflicting. At least oral antihistamines will reduce urticarial flare-ups [8]. Most antihistamines are probably ineffective, but in young children they are at least useful because of sedation. The newer generation cetirizine and loratidine are thought to be effective in AD and were recently investigated in clinical trials [14,15]. Cetirizine inhibited the in vivo and in vitro chemotactic response of T lymphocytes and monocytes [16].
There is also a lot of debate concerning the safety of antihistamines. We use dimetindene in the first year of life (after the age of 6 months) and, considering the low number of reported serious adverse advents. We have never observed serious side effects.

Antibiotics/Antiseptics

Topical or oral antibiotics or topical antiseptics are often used, although there are doubts on their usefulness. Therefore the role of antibiotics is conflicting. Some investigators believe in its use, but others do not.

Systemic treatment in recalcitrant disease

Oral cyclosporin can be considered in recalcitrant cases of childhood AD. The initial dose is 5-6 mg/kg daily and in some patients the dose can be reduced according to the response. Therapy should be continued for a maximum of 3 months. Therapy is well tolerated and has no side-effects in most patients [17], except the very rare increases in alkaline phosphatase [18]. Cyclosporin is safe and effective in short-term treatment for severe childhood AD [17]. Short-term treatment with cyclosporin may improve the long-

term outcome of AD, although most patients relapsed within 1-2 months after cyclosporin was stopped [19].

Elimination diets

Elimination diets are probably beneficial in some young children with AD. Many different opinions exist on food allergy and it may be considered a controversial issue. In our opinion it plays an role in 10-20% of the young children with AD with direct effects on eczema in about 4 of 10 cases [20]. Contact urticaria syndrome is one of the major manifestations of food allergy in small children with AD and food allergy [21,22]. Most commen allergens are eggs, cow's milk, peanuts, wheat and fish, but it also depends on consumption customs. Probably direct cutaneous contacts are important in inducing food and aero allergy. Not only immune-mediated reactions occur, also pseudo-allergic reactions such as with chocolate and oranges may occur. Oral food challenge should be performed cautiously in a hospital setting and is considered to be the gold standard for diagnosis. However, the criteria for oral food challenges need a defined cutoff point and standardization.

Other Therapies

Chinese herbs have been reported to be useful in therapy-resistant cases, however, side effects such as liver function disturbances have been reported [23, 24]. Besides, Chinese herbal physicians have combined this therapy with old-fashioned topical corticosteroids leading to plasma cortisol suppression.

Phototherapy is helpful in cases nonresponsive to local and systemic therapy; however, in general terms I would not advise giving it to children under the age of 18 years. In extremely recalcitrant cases combination therapy with UVA and UVB is indicated. Appropriate doses are 3-5 J UVA and 30-50 mJ UVB. Oral PUVA is used in older children with severe disabling AD with good results. Sheehan et al. [25] state that use of oral PUVA is justified in these serious cases, despite anxieties about possible long-term hazards. Highdose UVA1 (340-400 nm) is extremely useful, but the long-term effects are not known, and the treatment should not be used in children [21.

Experimental Therapy

Promising experimental treatment results with tacrolimus ointment and ascomycine ointment, drugs completely different from corticosteroids, have been reported. Most experience has been obtained with tacrolimus ointment. The therapeutic effect is seen within 2 days, and moderately severe AD can be controlled by twice weekly applications (personal communications). These drugs will become available in the future. These products are more effective than placebo and at least equal to moderately potent corticosteroids (Symposia Clinical Dermatology 2000 Congress, Singapore 1998).

Recombinant human interferon-y therapy may be effective in the longterm treatment of patients with AD [26]. The results are, however, conflicting.

High-dose intravenous immunoglobulin therapy in AD and hyper-IgE syndrome may be a potent, but very expensive alternative in the treatment of severe, steroid-dependent allergic disorders such as AD and allergic asthma [27, 28]. However, others have found it not to be effective in eczema [29].

In the distant future, new approaches for therapy of AD may involve the use of adhesion molecules or monoclonal antibodies directed against them and T-cell inhibitors [30].

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Fluticasone propionate 0.05% cream once daily versus Clobetasone butyrate 0.05% cream twice daily in children with atopic dermatitis

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Abstract

Background: Fluticasone propionate is a novel and potent corticosteroid. It seems to have an improved therapeutic index according to studies on skin thinning and suppression of hypothalamic-pituitary-adrenal axis.

Objective: To assess efficacy and safety of fluticasone propionate (FP) 0.05% cream once daily as compared with clobetasone butyrate (CB) 0.05% cream twice daily in children with atopic dermatitis (AD).

Methods: Twenty-two children (3 to 8 years old) with moderately active AD received either FP once daily or CB twice daily. Severity of AD was scored weekly using the modified scoring of atopic dermatitis (SCORAD) and treatment was either stopped when skin lesions were almost cleared (SCORAD <9) or after 4 weeks. Cortisol excretion was determined in 24 hours urine before and after treatment.

Results: Twenty-one children completed the study. After 1 week of treatment, mean SCORAD significantly decreased in both treatment groups. After 2, 3 and 4 weeks cumulatively 8, 12 and 16 children were clinically healed (SCORAD <9). No significant differences in the efficacy

were observed between the two treatments. Urinary cortisol excretion was not altered by either of the treatments (compared between groups and in time). Two weeks after discontinuation of active treatment, mean SCORAD had increased to 22, but still was significantly lower than that at the beginning of the study.

Conclusion: Once daily treatment with FP is as safe and effective as twice daily treatment with CB in children with AD. All of the children experienced an exacerbation of AD within two weeks, after treatment was withdrawn, indicating the need for long term "intermittent" treatment.

Introduction

Topical corticosteroids are the mainstay of therapy in atopic dermatitis (AD). Based on the vasoconstrictor assay[1], they may be divided into the following four groups: mildly potent, moderately potent, potent and very potent[2]. Usually, children with AD can be managed using emollients and mild to moderately potent topical corticosteroids. However, some children require further anti-inflammatory treatment. For this purpose, potent and safe topical corticosteroids are necessary. Until recently, it was generally assumed that the adverse effects of topical corticosteroids were directly related to their potency[2]. However, new corticosteroids have been developed with the promise of potent local efficacy and relatively little or no suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis[3,4]. Fluticasone propionate (FP) is a potent corticosteroid with such an improved topical/systemic activity ratio[5]. This is of particular significance in children who have a high ratio of body surface to body weight. In these patients systemic absorption is of major concern when potent corticosteroids are used.

Despite the widespread use of topical corticosteroids in children with AD there are surprisingly few clinical studies available on the efficacy and safety of topical corticosteroids in children. With respect to the use of potent topical corticosteroids in young children with AD, clinical studies are almost totally lacking. The scarcity of clinical trials in this field may be a result from the justified caution on the use of potent corticosteroids in young children. Another reason may be the opinion that the vasoconstrictor test[1], a ranking system for the potency of topical corticosteroids, would make clinical trials unnecessary. This opinion has been challenged

recently by the demonstration of a rather weak correlation between vasoconstrictor assay and clinical outcome[6], and by the development of topical corticosteroids with an improved benefit-risk ratio. Taken together, there is still a need for clinical trials.

Twice daily use of Clobetasone butyrate (CB) 0.05% cream, a moderately potent corticosteroid, is a well evaluated therapeutic regimen which was shown to be safe and effective in the acute phase treatment of atopic dermatitis in children[7,8]. Therefore we used CB 0.05% cream twice daily as the reference for comparing and evaluating the efficacy and the safety of FP 0.05% cream applied once daily in children with atopic dermatitis.

Methods

Patients and design

Twenty-two children (12 males and 10 females) with AD[9], aged 3 to 8 years were included. Disease severity was moderate severe according to the SCORAD[10] (SCORAD between 20 and 40). The patients had received no systemic treatment for AD in the month preceding the study. At the beginning a one week wash-out period with restricted medication (emollient, hydrocortisone acetate 1% and/or antihistamines when needed) was implemented. Thereafter, the children were randomized to receive either 0.05% FP cream once daily or 0.05% CB cream twice daily in a double-blind setting. To keep the study blinded, patients in the once daily group received vehicle cream in the morning and FP cream in the evening. During the trial all of the children used the same kind of basic skin care (emollient, bath oil). Visits to the center were planned once a week to assess the severity of AD (SCORAD) and the occurrence of side-effects. Treatment was stopped when SCORAD was below 9 (clinically healed) or ultimately after 4 weeks. A two- week follo- up period with basic skin care only (no anti-inflammatory treatment) served to evaluate the decline of AD and whether a rebound occurred. The study was approved by the local Medical Ethical Committee and written informed consent was obtained from the parents of all participating children.

Clinical scoring system

The severity of disease activity was scored using the modified Scoring atopic dermatitis (SCO-RAD) system[10] which is a composite index including the assessment of two items in a standardized manner: a) extent (applying the rule of nine) and b) intensity (erythema, edema/papulation, oozing/crusts, excoriation, lichenification and dryness). This scoring system reflects the modified consensus of the European Task Force on Atopic Dermatitis. Unlike the initial version of the SCORAD index[11], subjective symptoms were not included for the scoring of AD[10].

	Fluticasone propionate cream	*Clobetasone butyrate cream*
Age (yr)	4.9 ±1.7	4.1 ±1.1
Weight (kg)	19.3 ±4.8	17.5 ±3.8
Sex (male/female)	4/8	7/2
Initial SCORAD	29 ±6.2	32 ±5.6

Table I. Characteristics of the patients in the two groups.

	Fluticasone propionate cream	*Clobetasone butyrate cream*
Week 1	0	0
Week 2	4	4
Week 3	2	2
Week 4	3	1

Table II. Number of patients scored as clinically healed (SCORAD <9) in the course of the study.

Safety analysis

To assess the systemic load we measured the excretion of cortisol in 24-hours urine at the beginning (baseline) and the last day of the treatment period and at the end of the follow up. After following 5 patients, it was discovered, that the amount of collected 24-hours urine differed considerably between the visits. It was then decided to use creatinine levels as marker for estimating the reliability of the amount of collected urine. In 2 patients, the creatinine excretion for a particular visit was significantly lower than the average creatinine excretion measured over the other two visits. It may be assumed that at those particular visits not all the urine that was produced was collected. Correction for creatinine was performed in these two patients by increasing the measured cortisol excretion according to the extent of missing creatinine.

In six patients, the reliability of the amount of collected urine was not tested because data for creatinine excretion were not available.

Statistical analysis

The mean downward linear trend in time for the objective SCORAD and its difference between the two treatment groups was estimated and tested using a random coefficient model. The mean intercept was assumed to be the same for both treatment groups.

Cortisol excretion between the two treatment groups was compared using the Mann-Whitney test, and compared in time within each of the two treatment groups using the paired Wilcoxon test.

Results

Twenty-two children (12 males, 10 females; mean age 4.6 years, range 3 to 8 years) were randomly allocated to receive either 0.05% fluticasone propionate (FP) cream or 0.05% clobetasol butyrate (CB) cream. Twenty-one children completed the study (one drop out in the CB group due to varicella).
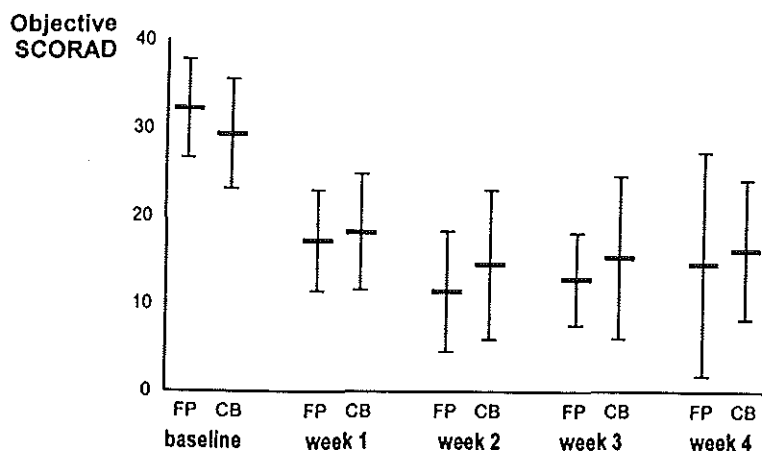
Figure 1. SCORAD (mean and standard deviation) for the groups treated either with 0.05% fluticasone propionate (FP) cream or 0.05% clobetasone butyrate (CB) cream. Note that patients scored as clinically healed (SCORAD <9) in weeks 2 and 3 are not included in the subsequent weeks. This results in a selection of poorly responding patients in weeks 3 and 4.
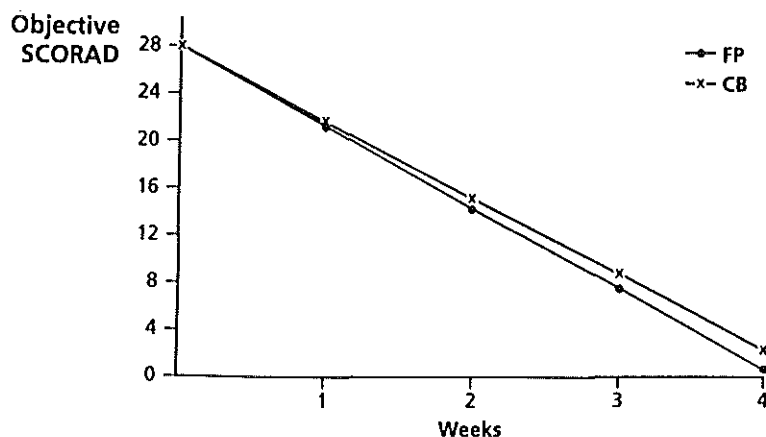

Figure 2. Mean downward linear trend in time of SCORAD in the goups treated either with 0.05% fluticasone propionate (FP) cream or 0.05% clobetasone butyrate (CB) cream. The difference in the slopes between FP and CB is not significant (p=o.8).

There were 12 patients in the FP group and 9 patients in the CB group. The two groups were well matched for age, weight and severity of AD. There was marked difference between the gender in the two groups (Table I). The initial SCORAD (mean ±SD) was 31 ±6 (FP: 32 ±6, CB: 29 ±6). As shown in Figure 1, after one week of treatment, the SCORAD (mean ±SD) significantly decreased in both of the treatment groups (FP: 17 ±6, CB: 18 ±6). In spite of the marked improvement after one week, none of the children was scored as clinically healed (SCORAD <9) (Table II). After 2,3 and 4 weeks respectively 8,4 and 4 children were scored as clinically healed (Table II) and active treatment was stopped.

At the end of the treatment period, the mean SCORAD had dropped to 10 ±10 (FP group) and 10 ±6 (CB group). Although the improvement of AD was more pronounced in the FP group, there was no significant difference in the mean downward linear trend of SCORAD between the FP- and CB-treated groups (Figure 2). After 4 weeks of treatment there were still 5 children with a SCORAD ≥9 (3 children in the FP group and 2 in the CB group). Two weeks after the discontinuation of active treatment, the mean SCORAD had increased to 23 ±9 (FP group) and 21 ±7 (CB group)

Urinary cortisol excretion was not significantly different between the two treatment groups at the beginning (P=0.8), at the end of the treatment (P=0.8) and at the end of the follow up (P=0.9). When comparing the 24 hours urinary cortisol excretion at the beginning with the excretion at the end of treatment (FP group: Z=-1.1, P=0.3; CB group: Z=-0.4, P=0.7) and at the end of follow up (FP group: Z=-0.4, P=0.7; CB group: Z=-1.2, P=0.2) there were no significant changes. However, there was one child in the CB group with an evident suppression of the urinary cortisol excretion after 4 weeks of treatment (Urinary cortisol excretion before treatment: 162.8 nmol/24hours; end of treatment: 67 nmol/24hours). At the follow up visit the urinary cortisol excretion had returned to the pre treatment value in this child.

Discussion

In the present study, a similar improvement was observed in two groups of children with atopic dermatitis who were either treated with 0.05% fluticasone propionate (FP) cream once daily or with 0.05% clobetasol butyrate (CB) cream twice daily. After one week of treatment all of the children experienced a significant improvement and at the end of the treatment period only one child out of the 21 children had not improved markedly.

As far as the literature is concerned, this is the first study on the topical treatment with FP in young children with atopic dermatitis. In adults and older children several double-blind studies, in which treatment with FP was compared with other topical corticosteroids have been published[12,13]. In one study involving adults and children above the age of 12 years with eczema, 0.05% FP cream applied twice daily was observed to have a similar efficacy to that of treatment with hydrocortisone-17-butyrate cream applied twice daily[12].
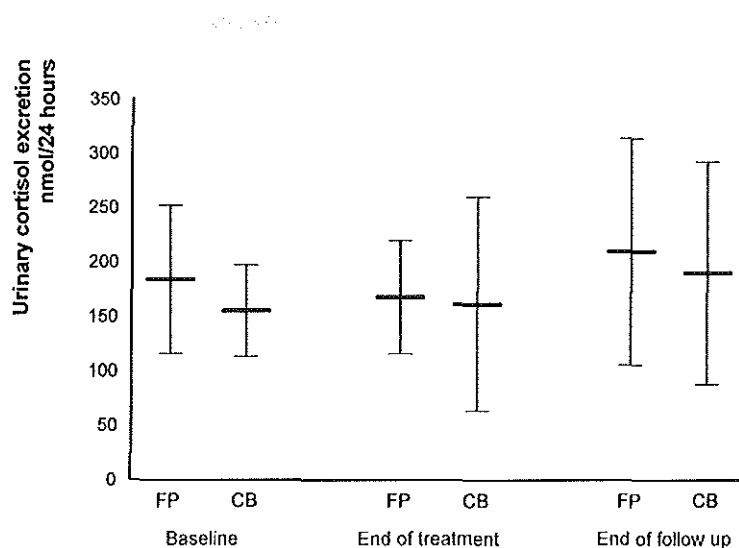

Figure 3. 24 hours urinary cortisol excretion (mean and standard deviation) for both treatment groups at the beginning of the study, at the end of the treatment period and at the end of the 2 week follow up.

In another double-blind study involving adults with atopic dermatitis, treatment with 0.005% FP ointment was compared with that using 0.05% Betamethasone-17,21-dipropionate ointment[13]. After twice daily application for 4 weeks, no significant difference in investigators overall assessment, symptoms (erythema, pruritus, skin thickening, lichenification, vesiculation, crusting) and patients assessment was observed. Regarding systemic side-effects, tretment with FP was noted to be safe as measured by plasma cortisol levels[12,13].

In the present study, we measured 24 hours urinary cortisol excretion. This turned out to be problematic due to incomplete urine collection. Therefore, creatinine excretion was determined and used for estimating the completeness of the urine collection. It turned out that most of the differing urine amounts could be attributed to real differences in urine production, whereas correction for creatinine was necessary in two children.

In agreement with other reports in adults[12,13], no significant suppression of the HPA axis after treatment with 0.05% FP cream was detected.

Besides systemic adverse effects, a number of local adverse effects have been associated with regular topical corticosteroid application. As far as clinical trials with Fluticasone propionate are concerned, burning (0-6%), folliculitis (1.7-4.3%), irritation (4%), hypertrichosis (1.7%) and pruritus (<3%) are the most frequently reported side-effects[13]. Skin atrophy is the most serious local side-effect. Age may be important, with younger patients experiencing more skin atrophy than older patients[15]. Data on the skin thinning effect of FP in children are not available. However, in a placebo-controlled volunteer study in adults, it was demonstrated that application of 0,05% FP cream once daily over a period of 8 weeks did not significantly affect skin thickness as measured by pulsed ultrasound and histology[16]. An explanation for the lack of atrophy observed in that study may be the unusual molecular steroid structure of FP based on the 19-carbon androstane structure. This structure is highly selective for the glucocorticoid receptor with little or no activity at progesteron, androgen, oestrogen, or mineralocorticoid receptors[4]. The half-life of the FP-receptor complex is 10 hours[17] which exceeds all other cor-ticosteroids. Although this indicates a high therapeutic potency, FP has been shown to have little effect on the HPA axis[5]. This may be due to limited percutaneous absorption and once in

the circulation, rapid elimination by liver metabolism (first pass effect approximating 100%). These properties suggest that FP is of particular value for treating young children, in whom systemic absorption is a major concern.

To date, data on the effect of fluticasone propionate in atopic dermatitis originate from studies in adults and cannot simply be extrapolated for use as guideline in children. The present study shows that 0.05% FP cream applied once daily is safe and at least as effective as 0.05% CB cream applied twice daily in the acute treatment of moderately severe atopic dermatitis in childhood. Further studies are imperative for evaluating the value of fluticasone propionate in the short- and long-term treatment of atopic dermatitis in young children.

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Treatment of erythrodermic atopic dermatitis with "wet-wrap" fluticasone propionate 0.05% cream/emollient 1 : 1 dressings

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Topical corticosteroids are the mainstay of therapy in atopic dermatitis. Recently corticosteroids with an improved benefit/risk ratio have been developed.[1] These show potent local efficacy and relatively weak or no suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis. Fluticasone propionate 0.05% cream or ointment is such a corticosteroid with an improved benefit/risk ratio.[2] Relatively few clinical studies on the efficacy and safety of topical corticosteroids in children are available.

In 1991, Goodyear et al described a method of treatment with dressings in children with erythrodermic atopic dermatitis.[3] Their method involves a wet-wrap technique using open-weaved cotton tubular dressings (Tubifast) impregnated with a 1 : 9 dilution (10%) of betamethasone valerate 0.01% applied twice daily according to a standard protocol.[4,5] This is done for a few days until 90-100% clearance of dermatitis is obtained. They have observed that 9 a.m. cortisol levels at the time of the wet wrap application are suppressed after 2-5 days, returning to normal 2 weeks post-therapy.[4] An attempt at long-term treatment with wet wraps in five patients at home was unsuccessful in all cases. Time-consuming application, decreasing effectiveness, secondary bacterial infection and prolonged HPA suppression were mentioned as reasons for failure.

We developed a modified protocol using fluticasone propionate 0.05% cream/emollient 1:1 diluted in the wet-wrap method (Table I). In contrast to the earlier reports we used a less diluted corticosteroid, applied only once daily. We also used the same in adult patients. We present the results in three children and four adults, all suffering from severe erythrodermic atopic dermatitis. The severity of atopic dermatitis was measured by evaluating the objective SCORAD.[6,7] A significant improvement in atopic dermatitis was observed after 2 weeks (SCORAD 74-38 to 33-10).

Table 1   Wet-wrap method: treatment procedure with fluticasone propionate (adapted from Goodyear et al[3] with modification)

1	Cut out appropriate sized pieces of Tubifast for the arms, legs, and trunk to form three separate suits.
2	Apply fluticasone propionate 0.05% cream 1 : 1 diluted with Vaseline 20% in cetomacrogol cream to the skin. In children older than 6 months and younger than 2 years the combination ratio is 1 : 3.
3	Soak the individual pieces of Tubifast in water.
4	Put on the first layer of 'wet' Tubifast. Tie the arm and leg pieces to the trunk section.
5	Apply a second dry suit of Tubifast over the top of the wet layer. Again tie the arm and leg pieces to the trunk section.
6	Make a mask for the face if involved. In patients younger than 2 years, apply hydrocortisone acetate 1% or clobetasol butyrate cream (both diluted 1 : 1 with emollient) to the face, soak the mask in water and put it on. Then apply a second dry mask of Tubifast over the top of the wet layer.
7	Re-wet the first layer every 2 h, and change daily after a bath (containing only oily bath additive).
8	After 2 weeks, apply fluticasone propionate 1 : 1 (or 1 3, depending upon age) diluted with emollient only on the active lesions 3-5 days a week and on the other days only emollient.

Suppression of the HPA, as assessed by measurement of serum cortisol levels in all seven patients, occurred in one adult patient during a 2-week period. However it normalized during the follow-up when measured 2 weeks later, while continuing the treatment. It is noteworthy that this adult female patient used the lowest amount of fluticasone propionate of all the adults (175 g in 2 weeks). She also used clobetasol butyrate and desoxymethasone on the scalp, and these could have also influenced the cortisol values. No suppression was seen in the remaining patients, while large amounts of fluticasone propionate were used, ranging from 95 to 555 g fluticasone propionate 0.05%, (Table II).

This therapy with a potent corticosteroid with minor systemic side-effects is an interesting and promising option in children and adult patients. In comparison with the earlier proposed

method, we used 50% corticosteroid versus 10% in the earlier studies, applied it only once daily versus twice daily and we used the same scheme with success also in adults.

Using the wet-wrap technique with fluticasone propionate 0.05%, we observed a striking improvement in erythrodermic atopic dermatitis within 2 weeks of treatment.

Of the seven patients (children and adults) treated, only one adult female had a temporary suppression of the HPA axis, but this patient also used other local corticosteroids. This method seems promising, especially with the use of local corticosteroids with only limited systemic side-effects. It is even more striking, because we used a dose of corticosteroid that was higher than used previously. Currently we are performing a study with different concentrations. A long-term study should establish whether this therapy is safe for prolonged use, free of complications and whether the patients are able to use it at home. The long-term follow-up in the patients described looks promising.

Table 2   Data of patients (3 children and 4 adults) with severe erythrodermic atopic dermatitis

| Patient | Age | Day 0 | | | Day 14 | | Amount of pure fluticasone propionate used[b] |
		SCORAD	Cortisol[a]	SCORAD	Cortisol[a]	
1	6 months	45	0.33	10	0.30	Unknown
2	4 years	38	0.33	18	0.22	95
3	6 years	47	0.40	10	0.23	130
4	35 years	74	0.21	33	0.23	555
5	36 years	64	0.37	30	0.37	300
6	44 years	65	0.24	18	0.10[c]	175
7	39 years	57	0.40	24	0.24	400

a Serum cortisol levels in μmol/l (normal range: 0.2-0.8 μmol/l)

b Amount of pure fluticasone propionate in g used in 14 days

c Also used 30 g clobetasone butyrate on face; after 6 weeks value returned to normal (0.24 μmol/l)

Chapter 5.3

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Efficacy and safety of Wet-Wrap dressings in children with severe atopic dermatitis: influence of corticosteroid dilution and duration of treatment

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Abstract

The wet-wrap treatment involves corticosteroid dilutions under occlusive wet dressings, and has been reported to be highly effective in severe refractory atopic dermatitis (AD). We investigated the influence of duration of treatment and different corticosteroid dilutions on the efficacy and hypothalamic pituitary adrenal (HPA) axis suppression in children with severe refractory AD. Eighteen children were treated with a 50% fluticasone propionate (FP) cream dilution for two weeks. In another 5 children a side to side comparison was conducted with 10%, 25% and 50% FP cream dilutions under wet-wrap, whereas a third group of 8 children was treated with 0%=emollient, 5%, 10% or 25% dilutions of fluticasone propionate cream applied on the whole body under wet-wrap. The systemic bioactivity was measured by the levels of 9 a.m. serum cortisol, 6 a.m. serum cortisol, and timed urinary cortisol/creatinine ratio. After one week, a major improvement was observed, without apparent differences between 5%, 10% or 25% dilutions of FP cream under wet-wrap. The improvement of AD amounted to 74% in the first and 8% in the second week of treatment. Furthermore, we noticed a significant dose response relationship for efficacy, with most cases being on the plateau of the curve, whereas the dose response curve for HPA axis suppression was without plateau. This indicated that less potent corticosteroid dilutions had comparable efficacy, but lower risk of HPA axis suppression as indicated by 6 a.m. serum cortisol levels. In conclusion, wet-wrap treatment is highly effective in severe AD. Less

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potent corticosteroid dilutions seem to have comparable efficacy but lower risk of HPA axis suppression.

Introduction

Severe atopic dermatitis (AD) remains difficult to treat. Although guidelines provide a good framework for managing AD,[1,2] some of the patients will not improve with the conventional therapy, posing a major therapeutic problem for the physician. Cyclosporin A, the most extensively studied regimen for refractory AD, was reported to be effective in children with severe AD.[3-5] However, cyclosporin A may have potentially severe side effects, and should not be given for longer than 3 months. In children, therapies like UVA-1,[6] PUVA,[7] recombinant interferon-γ[8] and systemic corticosteroids are not indicated. Thus, there is still a need for an effective treatment in children with severe AD.

In 1991, Goodyear et al described a highly effective method of treatment with dressings in children with severe erythrodermic atopic dermatitis.[9] Their method involved a wet-wrap technique involving the use of open-weaved cotton tubular dressings (Tubifast) impregnated with a 10% dilution of betamethasone valerate 0.01% cream applied twice daily according to a standard protocol.[9-11] They observed that 9 a.m. cortisol levels at the time of the wet-wrap application were suppressed after 2-5 days, but returned to normal 2 weeks post-therapy.

We developed a modified protocol using fluticasone propionate (FP) 0.05% cream-emollient dilutions (50% on the body and 10% on the face) once daily under wet-wrap dressings.[12] Fluticasone propionate is a potent corticosteroid, which seems to have an improved benefit/risk ratio[13,14]. In the first part of the study, involving 18 children, we observed a major improvement in all children and reversible 9 a.m. cortisol suppression in 3 children after 2 weeks of treatment. However, 9 a.m. serum cortisol is not a sensitive marker of the systemic bioactivity of topical corticosteroids, and other investigators used less potent corticosterod dilutions. Although the application of corticosteroids under occlusion was associated with a high rate of side effects in the past, the wet-wrap treatment is widely used, but the technique has not been satisfactorily evaluated. The purpose of this study was to investigate the efficacy and the safety of once daily wet-wrap dressings with different

fluticasone propionate dilutions and the duration of therapy in children with severe atopic dermatitis.

Materials and Methods

Patients and design

Thirty-one children (15 males and 16 females) with AD according to the criteria of Sampson[15] and Williams et al,[16] aged 5 months to 13 years were included. Disease severity was severe in 29 of the children according to an objective SCORAD $\geq 40^{17}$. The wet-wrap protocol we used was described previously.[12] Briefly, the patients were admitted to the hospital and took a bath for 5-10 minutes once daily, followed by the application of the diluted fluticasone propionate (FP) cream on the whole body. On top of this we applied first a wet and then a dry layer of tubular bandage made of cotton (tubifast[®]). This bandage was rewetted every two hours with water using a spray bottle.

The first group consisted of 18 children who were treated with a 50% dilution of FP cream under wet-wrap dressings for 2 weeks. The objective SCORAD and 9 a.m. serum cortisol were measured at the beginning and after 2 weeks of treatment.

The second group of 5 children had symmetrically localised AD and was treated with different dilutions (10%, 25%, and 50%) of FP cream on the left and the right side of the body under wet-wrap dressings. After 1 week, the severity of AD was scored by objective SCORAD on both sides, and treatment was continued with a 10% dilution of FP cream. The levels of 9 a.m. serum cortisol were measured at the beginning and after 2 weeks of treatment.

The third group consisted of 8 children who were treated with different dilutions of FP cream (0%=emollient, 5%, 10%, and 25%) under wet-wrap dressings in each of 2 children. Objective SCORAD and 6 a.m. serum cortisol were measured daily during the first week, and after 2 weeks of treatment. Treatment with topical corticosteroids was stopped 3 days before the baseline cortisol value was assessed, and the total amount of FP cream that was applied was recorded.

Clinical scoring system

The severity of AD was scored using the objective SCORAD[17,18] which includes the assessment of two items in a standardised manner: a) extent (applying the rule of nine) and b) intensity (erythema, odema/papulation, oozing/crust, excoriation, lichenification and dryness on a scale from 0 to 3). This scoring system reflects the modified consensus of the European Task Force on Atopic Dermatitis. Unlike the initial version called SCORAD index,[19] subjective symptoms are not included in the scoring of AD in the objective SCORAD[17,18].

Safety analysis

To determine the systemic load of the topical medication we assessed the HPA axis suppression. In the first 2 groups of 18 and 5 children respectively, 9 a.m. serum cortisol levels were measured at the beginning and after 2 weeks of treatment. In the third group of 8 children serum cortisol at 6 a.m. and urinary timed morning cortisol/creatinine ratio were measured daily during the first week of treatment. Local side-effects were assessed by visual investigation.
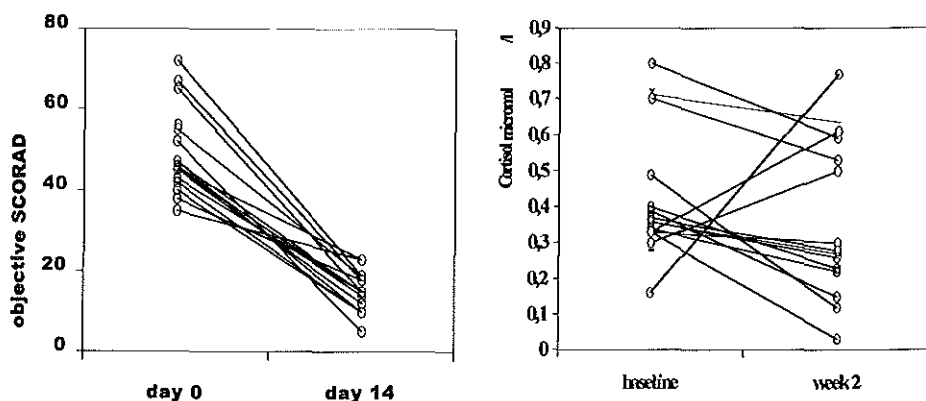
Figure 1. Graph showing symptom score and systemic load of wet-wrap dressings using a 50% dilution of FP cream in 18 children. A) Objective SCORAD before and after 2 weeks of treatment. B) 9 a.m. serum cortisol levels before and after 2 weeks of treatment.

Table 1   Number of children with mild, moderate,
                 and severe AD before and after treatment.

	Before treatment n=18	After treatment n=17
Mild AD	0	11
Moderate AD	2	6
Severe AD	16	0

Statistical analysis

Statistical analysis was performed with SPSS 7.5 using the Wilcoxon signed ranks test to assess differences in improvement of AD between the first and second week of treatment. The Spearman rank correlation was used to test monotonic relationships between improvement of AD, amount of FP cream / m^2 body surface, number of days with decreased serum cortisol level, and level of serum cortisol on day 7. Statistical significance was defined as $p<0.05$.

Results

In the first group of 18 children, a significant decrease in the objective SCORAD ($p<0.0001$) after 2 weeks of treatment was observed in 17 children (Fig.1 A). One child was lost to follow-up after 4 days of treatment due to personal reasons. The severity of AD classified into mild, moderate, and severe according to the objective SCORAD decreased in all 17 children (Table 1). For the evaluation of the safety we assessed the levels of 9 a.m. s

erum cortisol at the beginning of the treatment and after 2 weeks (Fig.1 B). Serum cortisol levels were temporarily below the normal range (0.2-0.8 μmol/l) in 3 out of the 18 children after 2 weeks indicating a suppression of the HPA axis.

Table 2   Objective SCORAD and extent of AD in 5 patients before and after one week of wet-
              wrap with different dilutions of fluticasone propionate on the left and the right side.

Patient	Left	Right
	Beginning ⇨ *after 1 week*	*Beginning* ⇨ *after 1 week*
A 10% vs. 50%	44 (65%)[#] ⇨ 9 (10%)	44 (65%) ⇨ 9 (10%)
B 25% vs. 50%	50 (55%) ⇨ 16 (26%)	50 (55%) ⇨ 16 (26%)
C 10% vs. 25%	55 (50%) ⇨ 13 (10%)	59 (30%) ⇨ 13 (10%)
D 10% vs. 25%	43 (60%) ⇨ 14 (10%)	43 (66%) ⇨ 14 (10%)
E 10% vs. 50%	40 (35%) ⇨ 12 (20%)	40 (35%) ⇨ 12 (20%)

The values in the table indicate objective SCORAD and the affected body surface in percent.
An objective SCORAD below 15 indicates mild AD, between 15 and 40 indicates moderate
AD and above 40 indicates severe AD.
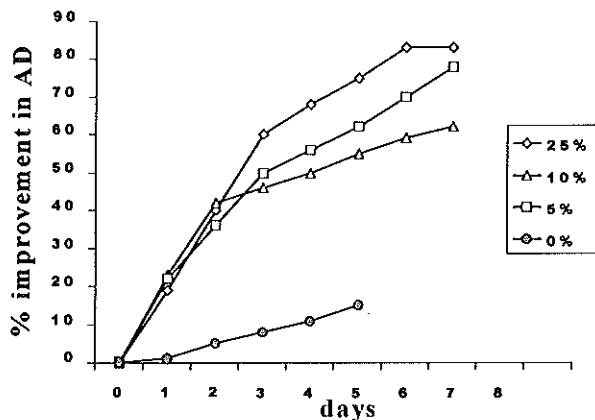
Figure 2. Graph of the relative improvement of AD in 8 children treated with different dilutions
(0%=emollient, 5%, 10%, 25%) of FP cream. The improvement of AD was measured by objective
SCORAD. Lines represent the means of 2 patients.
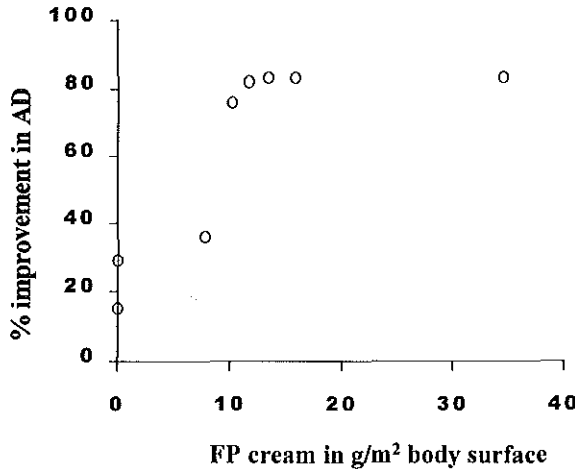
Figure 3. Graph showing the relative improvement of AD against the amount of FP cream / m^2 body surface in 8 children treated with different dilutions (0%=emollient, 5%, 10%, 25%) of FP cream.

To investigate the efficacy of different dilutions of FP cream under wet-wrap, a side to side comparison was performed in 5 children with different dilutions (10%, 25%, and 50%) of FP cream on the left and the right side. After one week of treatment, the objective SCORAD had decreased markedly in all 5 children, with no difference between the left and the right side in objective SCORAD or affected body surface (Table 2). Furthermore, we noticed that already after one week of treatment major improvement in AD was achieved, while the second week of treatment resulted in only minor additional improvement. After 2 weeks of treatment, we did not observe a decrease in 9 a.m. serum cortisol in any of the 5 children (mean serum cortisol day 0: 0.42 ± 0.16; day 14: 0.45 ± 0.17).

In the third group of 8 children, 3 different dilutions of FP cream (5%, 10%, 25%) were applied on the whole body in 6 children and emollient only in 2 children under wet-wrap . After one week, the 2 children using only emollient under wet-wrap showed minor improvement, whereas in the 6 children in whom using 5%, 10% or 25% dilutions of FP cream were applied under wet-wrap showed a major improvement in AD (Fig. 2).

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Apparently, there was no major difference in efficacy between 5%, 10% and 25% dilutions of FP cream (Fig. 2). However, a dose response relationship was obvious when the amount of FP cream/m² body surface was considered (Spearman's correlation r=0.97; p<0.0005) (Fig. 3). As seen in Fig. 3, there appears to be a plateau in the dose response relationship, indicating that amounts of FP cream above 12 g/m² body surface did not increase efficacy. Furthermore, we observed major improvement after 1 week, with little improvement in the second week of treatment (Fig 4). The difference in improvement between the first and second week of treatment was statistically significant (z=-2.02; p<0.05).

Adverse events, other than HPA axis suppression, in the first group were upper respiratory tract infection in 6, folliculitis in 6, herpes simplex infection in 1, diarrhea in 1 and itching in 1 out of the 18 children. In the second group of 5 children, we observed upper respiratory tract infection in 2, folliculitis in 2, abdominal pain in 1 and itching in 1 of the children. In the third group, we observed folliculitis in 5, balanitis in 1 and furunculosis in 1 of the 8 children. A generalised folliculitis was observed in both of the children treated with only emollient during the first week.

For a detailed analysis of the systemic load, 6 a.m. serum cortisol levels and timed morning urinary cortisol/creatinine ratio were measured daily in the third group of 8 children. A decrease in serum cortisol over the first week was observed and was the highest in the group treated with 25% FP cream, followed by the group treated with 10% FP cream and 5% FP cream (Fig. 5).

Furthermore, we noted a significant relationship between the number of days with abnormally low serum cortisol levels (<0.2 µmol/l) and the cumulative amount of applied FP cream /m² body surface (r=0.77; p<0.05). This indicated a dose response relationship for HPA axis suppression. The lowest levels of 6 a.m. serum cortisol were observed on day 4. However, no significant relationship was noted between the cumulative amount of FP cream /m² body surface and the 6 am cortisol level or urinary timed morning cortisol/creatinine ratio at day 7 (r=-0.46; p=0.26).
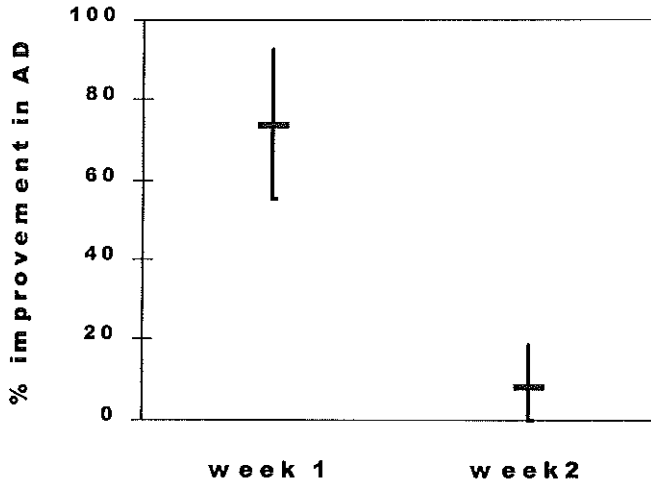
Figure 4. Graph showing the relative improvement (mean ±SD) during the first and the second week of wet-wrap treatment. The difference was statistically significant (z=-2.02, p<0.05)
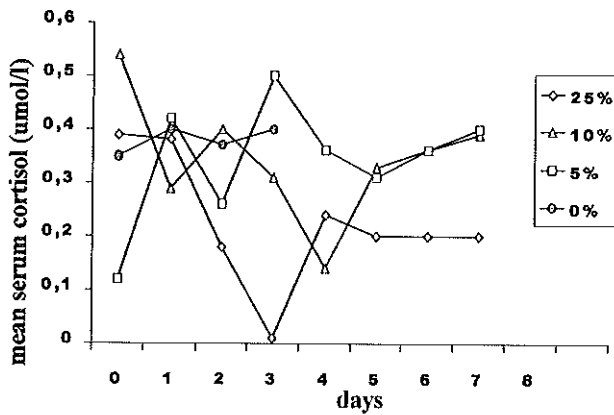

Figure 5. Systemic load as measured by 6 a.m. serum cortisol levels in 8 children treated with different dilutions (0%=emollient, 5%, 10%, 25%) of FP cream.

Discussion

The results of the present study show a dramatic improvement in AD irrespective of the dilution of fluticasone propionate (5%, 10%, 25%, and 50%) applied under wet-wrap. This is demonstrated both in a left to right side comparison in 5 children and by application of different dilutions in 8 children. The improvement of AD was significantly related to the amount of applied corticosteroid per m^2 body surface. This relationship was apparent in a dose response curve in 8 children, which was characterised by a plateau. Most of the cases treated with FP cream under wet-wrap were located on this plateau indicating that less potent dilutions of the corticosteroid would result in a comparable improvement in AD. Taken together these results indicate that less potent dilutions of FP cream under wet-wrap may have efficacy similar to that with dilutions of higher potency. The improvement in AD occurred mainly during the first week of treatment. During the second week of treatment minor additional improvement was observed indicating that one week of treatment with wet-wrap is sufficient.

Most of the side-effects during the first 2 weeks of treatment were probably not related to the treatment. Mild to moderate folliculitis, mainly on the legs, was observed in a large proportion of the children and appears to be related to the treatment. Interestingly, both children treated with only emollient under wet-wrap had generalised but mild folliculitis after 4 days. Thus, folliculitis under wet-wrap therapy may be related to the kind of occlusion constituted by the wet-wrap dressings. The addition of a corticosteroid may partly suppress the development of a folliculitis, but in some cases may aggravate the condition.

The systemic load was measured by morning serum cortisol levels. In the first and the second group of children, 9 a.m. serum cortisol levels were below the normal range (0.2 µmol/l) in 3 out of 18 in the first group and none out of 5 in the second group of children. However, 9 a.m. serum cortisol has been reported to have large intra- and interindividual variability, which makes it a rather insensitive screening method for the systemic bioactivity of topical corticosteroids.[20] Therefore, in the third group of 8 children, we determined daily 6 a.m. serum cortisol levels. The collection time is crucial as a three-fold difference in the levels of serum cortisol between 8 a.m. and 10 a.m. has been documented[21]. The early collection at 6 a.m. should minimize variations in cortisol levels. Additionally, urinary timed

morning cortisol/creatinine ratio was assessed, as this measure was reported to have excellent sensitivity[22,23]. By using these determinations we observed evidence of HPA axis suppression and we noticed a statistically significant dose response relationship between the amount of applied FP cream/m^2 body surface and the number of days with low 6 a.m. serum cortisol (<0.2 μmol/l). In contrast to the dose response curve for efficacy, the dose response relation for systemic bioactivity showed no plateau. This indicated that decreasing the amount of applied corticosteroid would improve the systemic safety without affecting the efficacy. Vice versa, increasing the amount of corticosteroid would increase systemic side-effects without increasing efficacy. Such a dose response relationship with dissociation between efficacy and side-effects is a documented attribute of most drugs. However, as there are no reference values for 6 a.m. serum cortisol or urinary timed morning serum cortisol/creatinine values, the clinical relevance remains unknown.

Remarkably, in the first and second group of children only 3 out of 23 children had evidence of HPA axis suppression, whereas in the third group of children all of the children had evidence of HPA axis suppression on at least one day. The discrepancy between these findings may be explained by the method that was used to measure the systemic load (9 a.m. serum cortisol versus 6 a.m. serum cortisol and urinary timed morning cortisol/creatinine ratio) and the timing of measurement (day 0 and day 14 versus daily assessment). Both measures are likely to increase the sensitivity of detecting the systemic bioactivity of a topical corticosteroid.

Using wet dressings for the treatment of an acute dermatitis is an old principle in dermatology. However, new is the use of fluticasone propionate, which is a corticosteroid combining potent local efficacy with relatively limited suppression of the HPA axis.[13,14,24] This improved topical/systemic activity ratio[25] is of particular benefit in children who have a high ratio of body surface to body weight and who may be at risk to develop systemic side-effects.[26]

In an earlier report, Goodyear et al reported that cortisol levels were uniformly low in all 30 children after 2 to 5 days of wet-wrap treatment. This is remarkable as they also used 9 a.m. serum cortisol, as in our first group of 18 children. The difference with our results may be explained by a different wet-wrap protocol. In contrast with the former publications we applied the wet-wrap once daily instead of twice daily and we used dilutions of fluticasone

propionate instead of betamethasone valerate on the body. We expected that the choice of fluticasone propionate may increase the safety of the wet-wrap. Furthermore, a twice-daily application is likely to increase systemic side effects. A recent review on the efficacy of once daily vs. twice daily applications of corticosteroids reported no evidence to support an advantage of multiple daily applications.[27]

No data are yet available on the efficacy and safety of long-term treatment with wet-wrap. We are currently investigating the wet-wrap for the long-term treatment in AD. Preliminary data reveal that a considerable number of patients failed to continue the treatment for a long time. Goodyear et al[9] reported that an attempt at long-term treatment with wet-wraps in 5 patients at home was unsuccessful in all cases. Time-consuming application, decreasing effectiveness, secondary bacterial infection and prolonged pituitary axis suppression were mentioned as reasons for failure. For the long-term treatment and guidance it is advisable to include regular nurse consultations in an out-patient setting.

The mode of action of the wet-wrap method is probably a combination of 1) protection of the skin from scratching 2) cooling of the skin and thereby reducing itching and inflammation and 3) enhanced penetration of emollients and corticosteroids. This increased penetration of corticosteroids is a potential hazard with regards to systemic side-effects. Therefore, regular monitoring of the HPA axis with a sensitive method is mandatory, particularly if the wet-wrap is used on the long term.

The present study is limited by the small sample size in which HPA axis suppression was investigated by means other than 9 a.m. serum cortisol levels, and can therefore not claim to provide reliable information on the safety of the wet-wrap treatment because susceptibility for HPA axis suppression is highly variable. The scope of the study was rather to compare different dilutions of fluticasone propionate under wet-wrap with regards to efficacy and systemic bioactivity.

We conclude that the wet-wrap therapy with fluticasone propionate cream is highly effective in severe and refractory AD. Furthermore, we conclude that a 5% dilution of the corticosteroid seems to have comparable efficacy, but lower systemic bioactivity than 10% or 25% dilutions under wet-wrap, and that one week of treatment is sufficient to achieve major improvement. Further study in a larger number of children is necessary to establish the

efficacy and the safety of less potent corticosteroid dilutions under wet-wrap dressings for long-term treatment.

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Chapter 6

Summary and discussion

Summary and general discussion

Atopic dermatitis (AD) is a chronically relapsing skin disease affecting about 10 to 20% of all children in western countries. Although, AD is often looked at as a minor skin disease, it has considerable impact on the child and its family even in mild disease.

In the experimental studies described in this thesis we investigated selected aspects of AD in children with a focus on evaluation and therapy. In order to study a disease, an appropriately validated system to score the activity of disease is essential. The SCORAD index and the objective SCORAD are widely accepted in Europe, but may be too complicated and time-consuming for daily practice. Therefore, in the study described in **chapter 3**, we evaluated a simplified system, the Three Item Severity (TIS) score, which is based on the evaluation of some selected items of the SCORAD, namely erythema, oedema/papulation and excoriation. We noted a fair inter-observer agreement for investigators using the TIS and we demonstrated a high correlation between the TIS score and the objective SCORAD. We concluded that the TIS is a simple and quick system to score the severity of AD in daily practice and to screen patients for inclusion in clinical trials on AD. However, this study had the same limitation as all other studies on scoring systems in AD: We have no information whether and to which extent the TIS score reflects therapy-related variations of the severity of AD as there is no biological marker that could be used as gold standard. Therefore, the TIS could not be related to an objective biological marker but only to another clinical scoring system, which remains an arbitrary selection of items for scoring.

In the studies described in **chapter 4** we investigated whether biological markers reflect the severity of AD by comparing their serum levels with the objective SCORAD. The levels of soluble adhesion molecules have been shown to reflect the expression of adhesion molecules on the endothelial cells and could potentially be used as an indicator of the inflammation in the skin. The correlation between objective SCORAD and the levels of potential markers of inflammation (soluble adhesion molecules, eosinophil cationic protein) and markers of atopy (total IgE, specific IgE) were investigated in studies described in *chapter 4.1*. We observed a highly significant correlation between the levels of soluble E-selectin and the objective SCORAD. This relationship was previously demonstrated in adults but has not yet been

described in children. We concluded that the levels of soluble E-selectin reflect the severity of AD both in adults and children and is a potential marker of inflammation in this disease.

In another study, described in *chapter 4.2*, we investigated whether the levels of soluble E-selectin, ICAM-1, and VCAM-1 reflect therapeutic interventions. Patients with severe AD were treated with the wet-wrap therapy, which resulted in a marked improvement after two weeks. We noted that the clinical improvement of AD was correlated with a significant decrease in the levels of sICAM-1 but not sE-selectin. This was unexpected because sE-selectin levels correlated with relatively small variations in SCORAD at the beginning, but it did not reflect the marked decrease in SCORAD after 2 weeks of treatment. These data indicated that the serum level of sE-selectin reflected the long-term (more than 2 weeks) severity of AD, but was not influenced by acute fluctuations in the severity of the disease. In conclusion, we advocate the use of both a clinical scoring system such as the objective SCORAD and additionally soluble E-selectin as an objective marker for the activity of AD.

The differential expression of T lymphocyte subsets, in particular Th1 / Th2 cells and their respective cytokines have been implicated in the pathogenesis of AD. Therefore, much of the basic research on AD concentrates on the T cell production of IL-4 and Interferon-γ. In the investigations described in *chapter 4.3*, we evaluated the DynaMIX DIA kit which is a recently introduced method for measuring the production of IL-4 and Interferon-γ in whole blood and we compared this method with the conventional ELISA technique. The DynaMIX DIA kit differs from the conventional ELISA in the options for running the assay using whole blood and for freezing material after stimulation. This results in time-saving and increased practical use. Using the manufacturer's instructions the DynaMIX DIA kit did not perform as well as the conventional ELISA. However, after adapting the culture and stimulation conditions, as described in our study, this system showed production levels of IL-4 and interferon-gamma similar to those obtained by our conventional ELISA. Based on its better practical performance the adapted DynaMIX DIA kit can be used effectively for assessing cytokine production.

Chapter 6 deals with the treatment of AD. An overview of the different aspects of therapy in children with AD is given in *chapter 6.1*. Guidelines have been established which provide a good framework for managing children with AD. In a recent consensus, emphasis was put on explanation and education of parents, use of emollients, the choice of the appropriate

corticosteroid and the avoiding provoking factors. In *chapter 6.2* the efficacy and safety of a new, so-called 4th generation topical corticosteroid, fluticasone propionate 0.05% cream was investigated in children with moderate active AD. Fluticasone propionate 0.05% cream is a potent corticosteroid which seems to have an improved topical/systemic activity ratio. We used clobetasone butyrate 0.05% cream applied twice daily as an established reference therapy. We observed that once daily application of fluticasone propionate 0.05% cream was highly effective and comparable to twice daily clobetasol butyrate 0.05% cream application. Local side-effects (striae, teleangiectasiae, skin thinning, hair growth) were not noticed and systemic side effects (temporary hypothalamic pituitary adrenal axis suppression), as judged by 24 hours urinary cortisol excretion, were apparent in 1 out of 21 children. This child belonged to the clobetasone butyrate group. We concluded that fluticasone propionate 0.05% cream was effective and safe for the short-term treatment of AD in children. After the corticosteroid treatment was stopped, we noticed recurrence of AD within 2 weeks, indicating the need for long-term treatment. However, reliable data on the long-term use of potent topical corticosteroids in children with AD are not available. With the experience obtained from this pilot study, a larger multi-centre study was planned with the aim to investigate both short- and long-term efficacy and safety of topical treatment with corticosteroids in children with AD.

However, in severe AD, which is refractory to conventional treatment as outlined in guidelines, other approaches are required. Severe AD remains difficult to treat. There is no consensus on the optimal regimen and the choice of treatment has to be individually determined. A modified protocol for the wet-wrap therapy in children with severe atopic dermatitis is presented in *chapter 6.3*. Basically, the wet-wrap therapy is an occlusive treatment using corticosteroids under tubifast dressings. Although, the wet-wrap therapy is widely used in some countries it has not been extensively studied. Safety considerations are crucial because such a therapy may be related to potentially severe side-effects. The presented data were based on a small number of children. Therefore, we investigated the efficacy and safety of the wet-wrap treatment in 18 children with severe atopic dermatitis. As described in *chapter 4.2*, we noticed a marked efficacy after 2 weeks in all children, but we also noticed systemic side-effects (reversible suppression of morning serum cortisol) in 3 of the 18 children. We concluded that the wet-wrap therapy was a very effective and

relatively safe treatment which, however, needs to be accurately followed in order to prevent clinically relevant side effects. The value of the wet-wrap therapy for the treatment of AD has to be considered in the context of other regimens for refractory AD like cyclosporine A or interferon-gamma, which also harbour the risk of potentially severe side-effects.

To increase the safety of the wet-wrap treatment, in *chapter 6.4*, we studied different dilutions of the applied corticosteroid with regard to daily serum cortisol levels and objective SCORAD. In this study, we observed a comparable improvement with different dilutions (1:3, 1:9, 1:19) of the corticosteroid. Furthermore, we noticed that virtually all improvement occurred in the first week. This study extended our knowledge on the optimal corticosteroid concentration and the optimal duration of daily wet-wrap treatment. As a result we adapted our wet-wrap protocol and at present we use once daily wet-wrap and less potent dilutions of fluticasone propionate for only one week. The safety of the therapy would therefore be increased.

In conclusion, the investigations described in this thesis deal with two aspects of AD in children: evaluation of the severity and therapy. For the evaluation of AD the TIS score may improve the way of recording in daily practice, whereas biological markers like soluble E-selectin may be a useful addition to a clinically based scoring system. For the treatment, this thesis offers new therapeutic modalities in both moderate and severe AD in childhood.

Summary - discussion

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Chapter 6

Samenvatting en discussie

Atopisch eczeem is een chronisch recidiverende huidziekte die 10 a 20% van alle kinderen in westerse landen betreft. Hoewel atopisch eczeem vaak als een milde ziekte beschouwd wordt, kan het grote invloed hebben op zowel kind als familie.

De experimentele studies in dit proefschrift behandelen de evaluatie en de behandeling van atopisch eczeem. Om een ziekte te bestuderen is een gevalideerd systeem voor het bepalen van de activiteit van de ziekte noodzakelijk. De SCORAD index en de objectieve SCORAD zijn breed geaccepteerd in Europa maar zijn te ingewikkeld en tijdrovend voor de dagelijkse routine. Daarom onderzochten wij in hoofdstuk 3 een eenvoudig meetsysteem, de "Three Item Severity (TIS) score". De TIS score is gebaseerd op de evaluatie van enkele criteria uit de SCORAD index, namelijk erytheem, oedeem/papels en krabeffecten. Wij vonden een goede overeenstemming tussen artsen die de TIS score gebruikten. Verder vonden wij een zeer goede correlatie tussen TIS score en objectieve SCORAD. Wij concludeerden dat de TIS score een eenvoudig en snel door te voeren systeem is om de ernst van atopisch eczeem in de dagelijkse routine te bepalen.

Deze studie heeft echter dezelfde beperking als andere studies over een op klinische factoren gebaseerd systeem voor het bepalen van de ernst van de ziekte namelijk: wij weten niet in hoeverre therapie gerelateerde variaties van de ernst van atopisch eczeem worden weergegeven door de TIS score omdat er geen biologische marker is die als gouden standaard toepasbaar is. Daarom werd de TIS score niet vergeleken met een objectieve biologische marker maar met een ander klinisch systeem, wat eigenlijk ook op een willekeurige selectie van symptomen berust.

In hoofdstuk 4 onderzochten wij de relatie tussen biologische parameters en de ernst van atopisch eczeem. Het is aangetoond dat de concentratie van "soluble" adhesie moleculen nauw gerelateerd is aan de expressie van adhesie moleculen op de celmembraan. Dit geeft aan dat de serum concentraties van adhesie moleculen gebruikt zouden kunnen worden als indicatie voor de uitgebreidheid en intensiteit van de ontsteking in de huid. In hoofdstuk 4.1. werd de relatie tussen potentiële biologische markers (soluble adhesie moleculen, eosinophil cationic protein, totaal IgE, specifiek IgE) en de objectieve SCORAD onderzocht. We zagen

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een duidelijk verband tussen de serumspiegels van soluble E-selectin en de objektieve SCORAD. Deze relatie was daarvoor al aangetoond bij volwassenen maar was nog niet beschreven bij kinderen. We concludeerden dat de serumspiegels van soluble E- selectin de ernst van atopisch eczeem weerspiegelen bij zowel volwassenen als kinderen en dat het een mogelijke marker is van de ontsteking bij de ziekte. In een andere studie, beschreven in hoofdstuk 4.2, onderzochten we of de serumspiegels van soluble E-selectin, ICAM-1 en VCAM-1 het effect van een behandeling reflecteren. Kinderen met ernstig atopisch eczeem werden met de "wet wrap" therapie behandeld. We zagen dat de klinische verbetering van atopisch eczeem geassocieerd was met een duidelijke afname van de serumspiegels van sICAM 1 maar niet met de serumspiegel van sE-selectin. Dit was onverwacht omdat relatief kleine verschillen in SCORAD gemeten voor behandeling wel correleerde met de spiegels van sE-selectin, maar de grote verschillen in SCORAD voor en na behandeling niet in een vermindering van de spiegels van sE-selectin resulteerden. Deze gegevens toonden aan dat de serumspiegel van s E-selectin de ernst op lange termijn (meer dan twee weken) weerspiegelt maar dat deze niet beïnvloed wordt door acute fluctuaties van de ernst van de ziekte. Concluderend adviseren wij het gebruik van zowel een op klinische factoren gebaseerd meetsysteem, zoals de objectieve SCORAD als ook de spiegels van sE-selectin als een objectieve marker voor de huidontsteking bij atopisch eczeem.

De differentiële expressie van subgroepen van T lymfocyten (T helper 1 / Thelper 2) en van de bijbehorende cytokinen worden in verband gebracht met het ontstaan van atopisch eczeem.

Daarom is veel van het basale onderzoek naar atopisch eczeem gericht op de T cellen en de productie van IL-4 en interferon-γ. In de onderzoeken beschreven in hoofdstuk 4.3, onderzochten we een recent geintroduceerde methode (DynaMIX DIA kit) om de productie van IL-4 en Interferon-γ in bloed te bepalen en we vergeleken deze methode met de conventioneële Elisa techniek. De DynaMIX DIA kit verschilt van de gebruikelijke ELISA methode door het gebruik van niet gecentrifugeerd bloed en de mogelijkheid materiaal tijdens de test te kunnen invriezen. Hierdoor is deze test eenvoudiger, praktischer en neemt de uitvoering minder tijd in beslag. Met de door de producent aanbevolen procedure presteert de DynaMIX DIA kit minder goed dan de conventionele ELISA. Echter, na aanpassing van de test wat betreft kweek en stimulatie vonden wij met de DynaMIX DIA kit

productie rates van IL-4 en interferon-γ die vergelijkbaar waren met degene van de conventionele ELISA. Wij concludeerden dat mits de kweek- en stimulatie methode aangepast wordt, de DynaMIX DIA kit een effectieve methode is om de productie van IL-4 en interferon-γ te meten.

Hoofdstuk 6 gaat over de behandeling van atopisch eczeem. In hoofdstuk 6.1 wordt een overzicht gegeven van de verschillende aspecten van therapie voor kinderen met atopisch eczeem. Er zijn richtlijnen opgesteld die een goed uitgangspunt vormen om kinderen met atopisch eczeem te begeleiden en te behandelen. Belangrijk is een goede uitleg aan de ouders, het gebruik van een emollient, de keuze van het juiste corticosteroïd en het vermijden van uitlokkende factoren. In hoofdstuk 6.2. is de effectiviteit en veiligheid van een nieuw zogenoemd 4de generatie lokaal corticosteroïd, fluticasone propionate 0,05% crème onderzocht bij kinderen met een matig actief atopisch exceem. Fluticasone propionate 0,05% crème is een sterk werkzaam corticosteroïd met, naar het lijkt, een verbeterde lokale versus systemische werking. Ter vergelijking gebruikten we een gangbare behandeling met clobetasone butyrate 0,05% crème 2 keer per dag aangebracht. We zagen dat het 1 keer per dag aanbrengen van fluticasone propionate 0,05% crème zeer effectief was en vergelijkbaar met het 2 maal daags aanbrengen van clobetasol butyrate 0.05% crème. Lokale bijwerkingen als striae,teleangiectasiae, verdikking van de huid en haargroei werden niet gezien. Systemische bijwerkingen die onderzocht werden in 24-uurs urine vonden wij bij 1 van de 21 kinderen. Dit kind behoorde tot de clobetasone butyrate groep. We concludeerden dat fluticasone propionate 0.05% crème effectief was en veilig voor de korte termijnbehandeling van kinderen met atopisch eczeem. Nadat de behandeling met corticosteroïden gestopt was, zagen we het exceem binnen twee weken weer terug komen. Dit is een aanwijzing dat een lange termijnbehandeling nodig is. Betrouwbare gegevens over de lange termijnbehandeling met sterke lokaal werkende corticosteroïden bij kinderen met atopisch eczeem zijn er echter niet.

Met de ervaring die uit deze pilot-studie verkregen is, werd een groter multicentre onderzoek gepland. Het doel ervan is om de effectiviteit en de veiligheid van lokale behandelingen met corticosteroiden bij kinderen met AD bij zowel lange- als korte termijnbehandeling te onderzoeken.

Echter, bij ernstig atopisch exceem wat resistent is tegen conventionele behandeling, is een andere aanpak vereist. Ernstig atopisch eczeem blijft moeilijk te behandelen. Er is geen consensus over de optimale benadering en de keuze van de behandeling moet individueel bepaald worden.Een aangepast protocol voor de wet wrap therapie bij kinderen met ernstig AD wordt beschreven in hoofdstuk 6.3. In feite is de wet wrap therapie een occlusive behandeling waarbij corticosteroïden gebruikt worden onder tubifast (katoenen verband). Hoewel deze therapie in sommige landen veel gebruikt wordt, is er nooit uitgebreid onderzoek naar gedaan. Controle van de veiligheid is zeer belangrijk omdat corticosteroïden onder occlusie ernstige bijwerkingen kunnen veroorzaken. De beschreven gegevens zijn gebaseerd op een klein aantal kinderen. Daarom onderzochten we de effectiviteit en de veiligheid van de wet wrap behandeling bij 18 kinderen met ernstig atopisch eczeem . Zoals beschreven in hoofdstuk 4.2 zagen wij een opmerkelijk verbetering van het eczeem na twee weken bij alle kinderen maar we merkten ook systemische bijwerkingen in de vorm van een reversibele onderdrukking van ochtend serum cortisol bij 3 van de 18 kinderen. Wij concludeerden dat de wet wrap therapie een zeer effective en relatief veilige therapie is die echter zeer goed gevolgd moet worden om klinisch relevante bijwerkingen te voorkomen. De waarde van de wet wrap therapie voor de behandeling van atopisch eczeem moet gezien worden in een context van andere regimes voor therapieresistent atopisch eczeem zoals cyclosporine A of interferon-gamma die ook een risico voor mogelijk ernstige bijwerkingen behelzen.

Om de veiligheid van de wet wrap behandeling te vergroten (hoofdstuk 6.4) bestudeeerden we de invloed van verschillende verdunningen van het lokale corticosteroid op de objektieve SCORAD en de serum cortisol spiegels. In deze studie vonden we een aanzienlijke verbetering van de ernst van het eczeem die niet duidelijk gerelateerd bleek te zijn aan de verdunningen (1:3, 1:9, 1:19) van het corticosteroid. Verder observeerden wij dat de verbetering van het eczeem zich voornamelijk voordeed in de eerste week. Deze studie vergrootte onze kennis omtrent de optimale corticosteroid concentratie en de optimale duur van de dagelijkse wet wrap behandeling. Als resultaat hiervan hebben wij ons wet wrap protocol aangepast en gebruiken op dit moment 1 maal per dag wet wrap met een hogere verdunning van fluticasone propionate voor de duur van slechts 1 week. De veiligheid van de behandeling is daardoor vergroot.

Concluderend kan gezegd worden dat de studies in dit proefschrift twee aspekten van atopisch eczeem bij kinderen behandelen: namelijk de evaluatie en de behandeling. Wat de evaluatie betreft kan de TIS score het begeleiden van een patient in de dagelijkse praktijk verbeteren, terwijl biologische markers zoals soluble E-selectin een zinvolle aanvulling kunnen zijn op een klinisch gebaseerd scoring systeem. Wat de behandeling van atopisch eczeem betreft worden nieuwe therapeutische mogelijkheden voor zowel matig tot ernstig atopisch eczeem voorgesteld.

Dankwoord

Het verrichten van onderzoek is vandaag vaak het resultaat van een samenwerking met velen. Voor een proefschrift geldt dit nog meer: Alleen kom je niet ver. Graag wil ik daarom aan het einde van dit proefschrift iedereen bedanken, die op welke manier dan ook, een bijdrage hieraan heeft geleverd. Allereerst dank ik de ouders en de kinderen voor hun deelname aan de verschillende studies. Verder wil ik een aantal mensen in het bijzonder bedanken.

Mijn promotor Prof. Dr. H.J. Neijens dank ik voor de vruchtbare en inspirerende discussies die wij over de verschillende onderzoeken hebben gevoerd. Hierbij heb ik geleerd de aspecten van mijn onderzoek kritisch van verschillende kanten te bekijken. Ook was ik altijd welkom als er iets besproken moest worden. De menselijke betrokkenheid bij mijn wel en wee was altijd een grote steun voor mij. Met name hiervoor bedankt.

Mijn co-promotor Dr. A.P. Oranje dank ik omdat hij de basis heeft gevormd voor dit proefschrift. Zonder zijn grote steun, vasthoudendheid en enthousiasme was dit proefschrift nooit tot stand gekomen. Hindernissen werden snel genomen. Dankzij zijn enorm enthousiasme voor het vak, de dermatologie, heb ik dit gebied van de geneeskunde leren waarderen. Nabesprekingen na een soms drukke en hektische poli waren nooit saai en gingen dieper en verder dan de huid van de patient. Ik heb mij thuis gevoeld op de dermatologie in het Sophia. Zijn nadrukkelijke lach die door de gangen van de poli klonk en het niet weg te denken strikje zullen mij altijd bij blijven. Bedankt voor de goede samenwerking.

Dr. H.F.J. Savelkoul, het enthousiasme waarmee je over cytokines en lymfocyten kunt praten is buitengewoon en besmettelijk. Discussies met jouw waren altijd prettig, inspirerend en leerzaam.

De overige leden van de kleine commissie, Prof. Dr. J.C. de Jongste, Prof. Dr. J.D. Bos, Prof Dr. P.C.M. van de Kerkhof wil ik bedanken voor hun bereidheid het manuscript kritisch te beoordelen.

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Curriculum vitae

Albert Wolkerstorfer werd op 11 augustus 1964 te Wenen geboren. In juni 1983 haalde hij het einddiploma op het "Musisch padagogische Realgymnasium". Hierna begon hij de studie geneeskunde aan de universiteit Wenen. Tijdens de studie volgde hij 2 maal een medical summerschool in Nederland. In juli 1991 over de "The dutch health care system" (universiteit Utrecht) en in juli 1994 over "pediatrics" (universiteit Groningen). Van januari 1993 tot juni 1994 deed hij onder leiding van Prof. M. Götz onderzoek bij kinderen met cystic fibrosis in het Academisch Kinderziekenhuis Wenen.

Hij promoveerde als "Doktor der gesamten Heilkunde" op 4 juli 1994 aan de universiteit Wenen. Hierna werkte hij 2 maanden als arts op de afdeling algemene pediatrie in het Academisch Kinderziekenhuis Wenen. Op 4 januari 1995 vond de beëdiging en registratie als arts in Nederland plaats.

Sinds 1 september 1995 deed hij wetenschappelijk onderzoek en poliklinische en klinische werkzaamheden op de afdeling dermatologie in het Sophia Kinderziekenhuis.

Per 1 juli 1999 is hij werkzaam als assistent geneeskundige niet in opleiding (AGNIO) op de afdeling kindergeneeskunde in het Sophia Kinderziekenhuis.