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Atopic Dermatitis

Editor

LASSE R. BRAATHEN

Contents

Preface

The third International Symposium of Atopic Dermatitis held 29.05–01.06.88 in Oslo followed the second symposium in Loen 4 years later. During these four years a very active work has been carried out all over the world in the field of atopic dermatitis and much new and exciting information was presented. The lectures held during the Symposium are included in this volume with exception for papers already published elsewhere or if the authors did not submit their papers for the present publication before deadline.

The editorial work has mainly been performed by Lasse R. Braathen. Apart from minor language corrections and alterations, the manuscrips appear here as they were submitted.

We hope this volume will reflect the advancement which has been achieved in the last years in research and in clinical aspects of the exciting and still so enigmatical skin disease.

Georg Rajka *Lasse R. Braathen*

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Atopic Dermatitis: a Love Affair

Memories and Reminiscences

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I fell in love with Atopic Dermatitis (AD) almost at first sight. We met for the first time in the summer of 1942 when I began my dermatology training at the Mayo Clinic. I was fascinated by the paradoxical extremes that occur in that disease: eczema in a person with the wheal and flare phenomena of atopy; vasoconstriction, facial pallor, erect nipples and erector pili contractions in a person whose trunk and extremities are reddened with vasodilation and inflammation; intense, tiny pruritic papules, some follicular and whealing, perhaps of cholinergic origin, when all else seems adrenergic and contracted: a tired face, older than its years, with infraorbital darkening and wrinkled skin in a person who is not yet 20 years old.

But most of all, I fell in love with the people who get the disease. When these individuals are well, they are bright-eyed, enthusiastic people relating well to others, being kind, helpful and cooperative. But when these individuals are sick they are miserable, resentful, hostile, selfish, uncommunicative and withdrawn. In the USA we describe this sick AD patient as having "face of a 'Wooden-Indian' ".

Let me explain: ... Tobacco and smoking was introduced to the Caucasian or so-called "civilized world" by the North American Indian and thus, the American Indian became the early symbol of the tobacco industry. During my grandfather's and father's lifetime the Tobacco Shops and Cigar Stores in the USA could be easily identified because outside of their entrances a statue of an American Indian, carved out of wood was sitting or standing; and sometimes holding a pipe or cigars. These statues are now only found in museums or in the shops for antique collectors. They are rare and quite valuable.

Let me show you one of these statues. I am certain that you, too, have had AD patients sitting before you who looked as cautious and reserved as this man ...

As you know, in 1892, AD was most comprehensively described by Besnier and even today, in Europe, "Prurigo Besnier" is an affectionate term that is still in use ... In 1902 Brocq added the name "Neurodermatitis Disseminata" ... And in 1933, although Sulzberger and Wise created the name "Atopic Dermatitis", which we now use, only he and a few of his NYC colleagues were using that term when I entered dermatology in 1942. At that time the official and accepted name in the USA was Disseminated Neurodermatitis, the same name that Brocq had introduced 40 years earlier.

This is understandable because in those days before the discovery of antihistamines and corticosteroids, the only long term approach to treatment, other than topical therapy, was a psychiatric one which we would now call "Behavioral Modification" since very deep probing of the psyche had to be a Freudian analytical approach which was not successful and sometimes even harmful to AD.

In the mid 1940s, a Dr Carl Menninger, one of America's finest psychiatrists, studied AD patients with us at the Mayo Clinic. At the end of one month he concluded that the severe AD itching was far too great a barrier to allow any proper psychiatric evaluation and treatment. What we needed, he exclaimed, was an anti-itch drug as effective and as specific as morphine was for pain. And, of course, we still do!

There was no question that the treatment and prevention of itch was our most difficult problem. It was already known that if the AD patients did not itch they usually would not scratch; and if they did not scratch they would not injure nor lichenify and sometimes would not even eczematize their skin.

In 1942 we always had at least 10–15 severe AD patients in the dermatology hospital at the same time. Their nocturnal scratching sounds were Symphonies of Percussion! ... The "rubbers" (those who rubbed their skin) made the caressing sounds of rubbing two pieces of leather together, the "scratchers" and "rakers" made the sound of scratching on sandpaper, the "patters and slappers" made the staccato notes of drumming and the "deep diggers" kneaded their skin like bread dough. Certainly it made one think that these different responses to itching may result from different types and causes of itch; a thought still kept in mind when treating AD.

Watching AD patients be suddenly seized by paroxysms of pruritus was reminiscent of the acute asth-

matic attacks which we had always stopped by injecting an intravenous bolus of aminophylline (a treatment first reported by E. P. Epstein in 1944). I gave that same treatment to these AD patients and I was pleased that it, too, aborted the paroxysms of pruritus. But at that time we also knew that we could do the same with an intravenous bolus injection of a 10% solution of Calcium Gluconate which made the patient gasp and feel warmly relaxed. We wondered whether we were merely distracting the patient with a bolus-type of injection because when we diluted the aminophylline and dripped it in slowly over a period of time there was no effect in treating or preventing the itch. Had we known then what we do now about the pharmacology of AD we would have persued that approach to treatment, but Cyclic-Amp, calcium binding proteins, etc., were not to be discovered for many years.

In the early 1950s I was trying to atropinize a local, 5 centimeter area of uninvolved skin on a patient with AD; I did this when we were studying the acetylcholine "delayed-blanch" phenomenon. But, I injected too much atropine intracutaneously and temporarily atropinized an AD patient who had been in remission; i.e., I induced a systemic atropinization reaction causing tachycardia, hyperventilation, severe agitation, restlessness and a diffuse, generalized, intense erythema. Previous to this moment the patient had been in a 3 month remission without any dermatitis, itching or even white-line phenomena. He immediately scratched & clawed himself all over because of intense itch. The "white-line" and the acetylcholine "delayed-blanch" reactions re-occurred everywhere on his red skin and persisted for the time it took for the atropine reaction to completely stop. Twenty four hours later the patient and his skin were back to the pre-atropinized, normal state. But with the neuropharmacology knowledge of 1951 we could not rationalize what we had done.

About 5 years later, reserpine (extracted from a rauwolfia plant) was introduced for the treatment of hypertension. Reserpine, like guanethidine, depletes stores of catecholamines in tissues, causing bradycardia and a decrease in vascular peripheral resistance, increased cutaneous blood flow and thus postural hypotension. It also causes central sedation and indifference. Side effects include flushing, nasal congestion, abdominal cramps and diarrhoea. Reserpine was also suggested for the treatment of AD. So, Dr Otis Jillson and I decided to prescribe it. But, as we always did with a new drug, Jillson and I first took the reserpine outselves in order to understand how the patient would feel and react. It takes several days for reserpine to achieve a therapeutic effect. Certainly our moods gradually flattened, but at the same time our noses became stuffy and all of our skin flexures warmed, became red and mine even itched (I think I am an unerupted, red-faced atopic and I am sure that I carry that gene). Then we gave the reserpine therapy to three patients with mild AD, who were friends and professional colleagues and interested in its possible benefit.

Unfortunately there not only was no benefit, but within a week, all three patients had severe, generalized flares of their AD. It took a month of intensive topical therapy before we could bring them back to a pre-reserpine state. By then, we had suspected that acetylcholine stimuli and cholinergic emotions could flare AD. But we were not clever enough to recognize that we, personally, had experienced acetylcholine-like effects from the reserpine. Later, in retrospect, we wondered if that was why our AD patients were made worse by it.

Subsequently, I have had several former AD patients, who were well into middle age and had lived decades without any dermatitis, suddenly develop AD again after receiving reserpine treatment by family doctors for "stress" hypertension. Stopping the reserpine always stopped the dermatitis.

Recall that AD patients are hypotensive at rest, but they respond with hypertension to the "cold-pressor" stress test! This was first described by Eyster, Roth and Kierland in 1952 when we were all just beginning to define a few of the physiologic and pharmacologic AD paradoxes; acetylcholine's "delayed blanch" was another. But we did not know how to interpret these cholinergic and adrenergic responses, nor how to interpret the blocking or enhancing effects of drugs such as atropine, nicotine, reserpine, histamine, etc., some of which I have already mentioned. Cyclic AMP & GMP, were unknown to us, then, as were cellular and humoral immunity, IgE, T-cells and B-cells, etc. They were yet to be discovered ... And we knew that allergy was somewhere in the picture because we had also seen AD patients made worse with antigenic vaccines used to treat their concomitant atopic asthma and rhinitis. But we did not know where to fit it into the puzzle ... And although we knew that atopy "ran in families" the sciences of modern genetics were just beginning to evolve.

Then came the 1960s when we were inundated with new knowledge in all these basic sciences areas. And

we tried, each in one's own way, to apply the excitement of these new concepts to AD ... It was not until 1968, when Szentivany's hypothesis suggested atopy might be a disease of defective beta-adrenergic receptor function, that the door was opened for many of your own accomplishments in the 1970s.

Now, in the 1980s we all know, for example, that there is an excess of phosphodiesterase and hyper IgE in active AD. And we know that many other pieces of new information from many different scientific areas are being added, by you, to solve the AD puzzle (e.g.: Type I Immunology, Cell Mediated Immunity, B and T cell interactions, the role of macrophage monocytes and Langerhans cells, cellular genetics, etc.). But where they fit and how they affect each other is not yet completely clear.

And now as we ... No, I should not say 'we' ... And now as '*You*' move into the 1990s let me assure you that I (this 76 year old 'lover' of Atopic Dermatitis) am very excited and I am assured and therefore quite content that all of you, the younger AD 'lovers', 'wooers' and 'courtiers', will make the complex AD facets interrelate, fit and fuse together into a clearer picture; also, that 'Your' students, the AD courtiers of the future, will carry on into the 21st century, putting the finishing touches to the portrait of our loved one, Atopic Dermatitis!

Thank you very much for allowing me to join you here in Oslo, to reminisce a little and to wish you well!

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On Definition and Framework of Atopic Dermatitis

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Based on experience in connection with working and discussing differential diagnostic problems, a definition of atopic dermatitis (AD) seems to be needed. This should include the relation to atopy: it is obvious that the person developing this skin disease is an atopic individual. At the same time it is of importance to emphasize that the ensuing skin inflammation is of a specific character, since atopic persons may also develop skin lesions which have no relevance to their genetic constitution and immunological behaviour. When defining the special traits of AD, the prominent and clinically basic symptom, the itch and subsequent morphological changes should be mentioned in first hand, whereas a delayed-type skin reaction, based on cellular infiltrate and immunobiochemical alterations should also be considered. The term of eczema is much debated (1) and in this case a too broad concept and not covering all morphological events. It is, however, deeply rooted in the dermatological literature, and, as compromise, "eczematous inflammation" is mentioned (see table I).

Secondly, a pathomechanistic model of AD is presented. There are several designs in the literature which summarize the different events in the mechanism of AD (2–7), or covering some aspects of it, like the events on the cell level (8). The aim of presenting a new model is to attempt to put the, especially from the clinical point of view leading trait of the disease, i.e. the itch, in the center of the pathomechanistic pathways. The design considers genetic as well as environmental factors and consequences of the impaired immunoregulation including biochemical alterations, changes in cell functions and the release of inflammatory mediators. As a consequence of the atopic state the production of higher IgE levels, elicited by different allergens occurs in the majority of the cases, resulting in type-I hypersensitivity manifestations. Mostly attributed to alteration in the sub types of T cells, a paradoxical situation emerges: while deficiency of suppressor and cytotoxic T cells leads to impairment of cell-mediated immunity with its important consequences, predominantly T helper cells, in cooperation with antigen-presenting cells, including Langerhans cells, and other cells, create the infiltrate, typical for a delayed-type response.

On the other hand, I want to emphasize that the inflammation, elicited by immunological alterations occur in a skin which is impaired in several of its functions; in other words: the atopic events appear in an abnormally reacting skin. These functional alterations of the skin in the AD patient include changes in sebum production, sweat inhibition, barrier function and result, among others, in increased staphylococcal colonisation and in general reduced resistance to contact irritants. It has been shown, that, compared to the skin of nonatopic persons, alterations are present in the non-lesional skin of patients with AD, although it is clinically symptom-free

The only obvious common link between the immunological and non-immunological traits, i.e. itch, should be put in the center of the pathomechanistic events when designing a model. Itch is also a typical sign in both type-I and type-IV responses and is their important clinical consequence (another link may be the Langerhans cell (9), see Table 2).

A further intention was to discuss which of the etiological or provoking factors are of primary or secondary character in the mechanism of AD. The use of the term "primary" is rather problematic, since it frequently may be assumed that there exists a preceeding factor or event to a fact which is called "primary". Thus I have chosen to make a distinction between events influencing or depending on the course of AD. In the latter case, the changes following the clinical course are obviously a consequence of the intensity of the clinical phase of the disease. In some details, the data do not yeat allow a classification into these principles and these aspects are symbolized with a question mark (Table 3).

Table 1. *Definition of AD*

Atopic dermatitis is a specific dermatitis in the abnormally reacting skin of the atopic resulting in itch with sequelæ as well as in eczematous inflammation

Table 2. *Mechanism of AD: attempt to synthesis*

cAMP = cyclic 3,5,-adenosinemonophosphate, PDE = phosphodiesterase, PML = polymorph leukocytes, Mono = monocyte, IgE/s = IgE in serum, Ts = suppressor T cell, Th = T helper cell, Tcyt = cytotoxic T cell, Teff = T effector cell

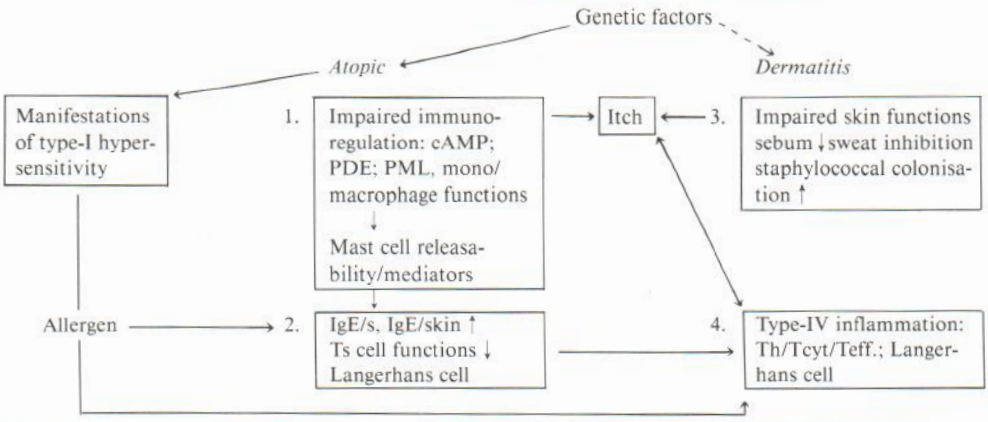

Table 3. *Putative primary and secondary immunological and pathophysiological events in the mechanism of AD*

Putative primary events (influencing the course of AD)	Putative secondary events (dependent on the course of AD)
1. Increased itch	1. Itchy skin
2. Increased PDE activity	2. High IgE production
3. Dry skin/reduced lipid secretion, Str. corneum alteration/increased TWL/reduced water content/ impaired barrier function, lower resistance to irritants, increased staphylococcal colonisation	3. Reduced cell mediated immunity
4. Mast cell releasability (?)	4. Reduced antiinfectious resistance/ reduced chemotaxis
5. Disturbed metabolism of linoleic acid in serum lecithin (?)	5. Vascular disturbances
	6. Sweat disturbances (?)

(?) = possibly pertinent to the other group.

Table 4. *Synopsis for the clinician*

Most stimuli either on allergic or nonimmunological basis reaching the skin outwards or inwards (incl. emotional influences) elicit or maintain *itch*

We have an immunological *imbalance*; therefore:
- Low defense against living agents
- Many positive immediate reactions with variable clinical significance

We have a *dysfunctioning* skin, therefore:
- The skin is dry
- Overcolonized with staphylococci
- Sweating leads to itch

Lastly, an ambition is mentioned in order to explain in a short and simple manner the important clinical consequences of the complex theoretical aspects of AD for the clinician, which he/she in turn can then point out for their patients (Table 4).

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Grading of the Severity of Atopic Dermatitis

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A simple system for baseline grading the severity of atopic dermatitis in clinical work, is presented. The grading, which may be carried out on the basis of one single consultation, permits distinction between mild, moderate and severe atopic dermatitis by means of a score summation using the following parameters: 1) extent (by "rule of nine"), 2) course (via history) and 3) intensity (disturbance of night's sleep by itching). ***Key words: Atopic dermatitis; Severity; Grading system.***

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In the literature the activity of the skin disease in patients with atopic dermatitis (AD) is estimated in rather different ways (1, 2, 3). In order to be able to compare results from different studies, it would be an advantage if a more uniform system was used for the definition of the severity of AD in clinical materials.

There are two major aspects of grading the disease activity in AD. The first one, which will be dealt with in this paper, is the distinction between patients with mild, moderate or severe disease activity, i.e. categorizing patients for clinical records. The second one concerns the recording of variations of disease activity, e.g. during a clinical trial of a new drug.

The aim of this paper was to work out simple criteria appropriate for clinical work, which could enable us to assess the severity of AD on the basis of a single consultation.

The present criteria for grading the severity of AD have been worked out on the basis of our earlier experience (4, 5, 6, 7) and discussion at a conference with invited experts, held at The 3rd International Symposium on Atopic Dermatitis (June 1, 1988 in Oslo) (Table I).

COMMENTS

The patient's history during the last year should be considered in order to correctly evaluate the actual clinical state—which may be exceptionally good or bad.

The "rule of nine" division of the body surface, widely used for classification of burn injuries, has been used as a practical way for estimating the extent of the dermatitis (Table II).

Due to a more widespread pattern of the dermatitis in infants than in older patients, other figures have been used to estimate the score for this parameter in infants.

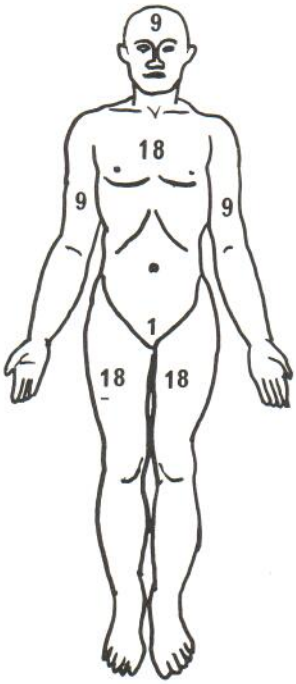
The course is judged according to the length of the remission periods. In infants or in cases where the

Table I. *Grading (severity) of atopic dermatitis*

I. *Extent*	
(*a*) Childhood and adult phase	
Less than approx. 9% of the body area	1
Involvement evaluated to be more than score 1, less than score 3	2
More than approx. 36% of the body area involved	3
(*b*) Infantile phase	
Less than approx. 18% of the skin involved	1
Involvement evaluated to be more than score 1, less than score 3	2
More than 54% of the skin involved	3
II. *Course*	
More than three months of remission during a year[a]	1
Less than 3 months remission during a year[a]	2
Continuous course	3
III. *Intensity*	
Mild itch, only exceptionally disturbing night's sleep	1
Itch, evaluated to be more than score 1, less than score 3	2
Severe itch, usually disturbing night's sleep	3
Score summation	
3–4 = mild	
4.5–7.5 = moderate	
8–9 = severe	

When doubt, score 1.5 or 2.5, may also be used.

[a] May be adjusted in infants or if onset was less than 1 year before grading.



disease first started less than one year ago, these criteria should be adapted to the actual period of disease.

Regarding the intensity of the disease, our approach is to use the itch as the parameter, as we consider this as the basic trait of AD, being the main complaint of patients with AD. According to our experience, the patient's complaints from itch will usually reflect the degree of the characteristic lesions of AD, such as scratch marks, prurigo papules and lichenification. Using the patients experience of itch, instead of recording various types of skin lesions, permits an assessment of the score for intensity based upon the history. Recording various skin lesions in order to assess the score for the intensity of the dermatitis, would have required several consultations in periods with various activity of the dermatitis.

It has been proposed that diary cards could be used in order to get more precise information about the disturbance of night's sleep due to itch. This would probably be of some help, but a problem is that this will require a period of time before the grading can be performed.

Table II. *Calculation ("rule of 9")*

	Percentage
Head	9
Upper extremity	9
Body, anterior	18
Body, posterior	18
Lower extremity	18
Genital area	1

Obviously, the actual clinical state is influenced by several factors, for instance by therapy or intensive staphylococcal colonization of the skin (5). Since most patients are treated with various topical preparations, we find it most appropriate not to adjust the scores for topical therapy, whereas adjustment of scores should be done in severe cases where the activity of the disease is suppressed by oral steroids or intense photo/photochemotherapy. Staphylococcal colonization, being present in most cases of severe AD (8), is considered as an aggravating factor for the development of AD. Therefore, adjustment of the scores seems not to be necessary with respect to the presence of this bacterium.

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Atopic Dermatitis: Elements in Clinical Study Design and Analysis

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Considerations in design and analysis of clinical trials in atopic dermatitis are discussed. Since studies analyzed statistically provide an impression of the "probable" effect on the "average" patient, the value of the conclusions depends on the limits imposed by the investigator(s) on factors such as sample size, heterogeneity of the patient population, relevance of the parameters measured and biases introduced in data collection and management. Suggestions are provided for inclusion/exclusion criteria, variables to be measured, sign/symptom scoring systems and data presentations in studies involving patients with atopic dermatitis.

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Clinical trials can be conducted for many reasons; the success of any trial initially depends on a clear definition of the objective and ultimately on the use of proper techniques to achieve the stated goals. Studies can be conducted to gain an early clinical impression of the efficacy of a treatment. In such cases, small populations and uncontrolled designs can be adequate. While the results from such trials may provide the rationale to pursue, discontinue, or modify a specific compound, small sample sizes and nonstatistical design usually preclude generalizations of the results to larger populations. The results will illustrate how the drug will affect the disease in specific patients under certain conditions. Conclusions will depend heavily on the attitude and experience of the investigator and the specific patients placed in the study.

In the development of drugs for market, or comparisons of different treatments, study designs must be developed which will provide data that can be extrapolated to large populations. The conduct of such trials requires relatively large sample sizes plus sophisticated statistical design and analysis. Conclusions based on such results/analyses will provide an impression of the "probable" effects of a new drug on the "average" patient. The results of these trials, however, are meaningless unless the limits of generalizations are set forth in detail. This paper addresses some of the elements of design and analysis which are important in defining these limits, especially as they pertain to the study of drugs for atopic dermatitis.

CLINICAL TRIAL DESIGN

Clinical trials whose results are to be generalized should be randomized, controlled and blinded and the objective should be clearly stated and not overly complex. Parallel group designs usually are required by the Food and Drug Administration in the USA for studies substantiating the safety and efficacy of a treatment; bilateral paired comparison designs are useful in early studies and in some comparative trials. Within this broad framework many types of designs can be employed (see, for example (1, 2)). During the initial considerations of study design, an estimation of the sample size required to achieve the objective should be made. Use of inadequate numbers of patients can lead to uninterpretable data and/or erroneous conclusions. Statistical methods exist which can provide estimates by accounting for the expected variability in the data, the possible magnitude of the differences between the treatments being compared, and the possible numbers of patients who might drop out of the study or be invalidated (3).

The use of multiple study centers helps to prevent a bias introduced by any single investigator and often is necessary in order to provide an adequate number of patients. However, a balance must be achieved between the use of too many centers, which may increase variability, and the use of too few centers, which may decrease generalizability. The use of an odd number of centers provides for a "tie-breaker" if data trends from different centers are contradictory, and scattering the centers over diverse geographical regions and environments helps to offset effects of climate, environment or culture.

Once the study objectives have been established, the variables which might affect study outcome should be defined and decisions made as to what limits, if any, should be imposed to increase the accu-

Table I. *Considerations in clinical trial design: minimizing variability/increasing definition*

Consideration	Control measure
Characterization of condition	Inclusion/Exclusion criteria
Control of concomitant disease/treatments	Inclusion/Exclusion; Restrictions
Evaluation of patient "demographics"	Inclusion/Exclusion; Data collection
Control of dosage form, regimen, duration	Protocol design
Evaluation/control of environmental factors	Protocol design; Data collection
Control of protocol adherence	Instruction; Information cards; Reminder diaries; Drug accountability
Definition of goal	Protocol design

racy and interpretability of the data. Tables I and II list some of considerations in the design of a clinical trial which help to define the limits of the indication/population being studied. The literature abounds with arguments on the pros and cons of carefully defined populations since "overselection" could introduce a bias into the study; however, without some sort of control, data generated will be impossible to pool and treat statistically. If different subsets of the population are expected to react differently to a given treatment—e.g., pediatrics vs adults, stable vs. flaring patients—subject stratification procedures can be employed during enrollment and randomization. However, this tactic is useful only if sufficient patients are available in the different strata to permit meaningful analyses. Unique subsets of the population always can be studied at a later time in separate studies.

Table II. *Critical inclusion/exclusion criteria in atopic dermatitis*

Clear diagnosis of atopic dermatitis, present at least one year
Current flare stable or slowly worsening for more than one week
Lesions suitable for evaluating response to test agents: severity of disease at target site must be such that the total of the numerical ratings for erythema, induration, pruritus is at least 5 out of a possible 9, with *all* parameters being present (rating scale 0=none to 3=severe)
No abnormal clinical, physical or laboratory findings
No hypersensitivity to test medications
No history of alcohol or drug abuse
No concomitant medications during study such as retinoids, antibiotics, large doses of antihistamines, tranquilizers, tricyclic antidepressants
Suitable wash-outs for retinoids, experimental drugs, corticosteroids

Table III. *Definition of efficacy in atopic dermatitis (sign/symptoms)*

Severity on 4 point scale (0=none to 3=severe; half values permitted)
Target area for close observation
Key signs/symptom (erythema, induration, pruritus) *all* must be present in target area; others (lichenification, vesiculation, crusting, oozing, scaling, etc) evaluated as applicable
Global evaluation for total picture (e.g., 0=100% resolution; 1=75% to 99% clear of signs and symptoms; 2=50%–74% clear; 3=25%–49% clear; 4=<25% clear; 5=exacerbation)

Decisions must be made even in the early stages of protocol design concerning data collection and expression. If such considerations are left until the study is completed, important information may be lost inadvertently. The primary variables to be studied must be defined and the manner in which they will be assessed must be determined (Table III). For topical drugs in atopic dermatitis, we generally specify target lesions on which close observations of signs and symptoms will be made; a global evaluation is used to account for changes in the overall condition of the patient's disease. We define the most critical and common signs and symptoms of disease as key signs and symptoms; these must be present in the target area of all patients. Tables IVA and IVB illustrate the prevalence—and severity—of various signs/symptoms during two separate studies of patients with atopic dermatitis (4). In both studies, the most common, and key signs/symptoms, were erythema, pruritus and induration. All others—lichenification, vesiculation, crusting, oozing and scaling—occurred with various frequencies among the population. Since study of these signs/symptoms could be informative, we evaluate them in the target lesion, collect the data, and analyze it as a supportive measure. The lack of adequate numbers of subjects with these signs/symptoms often precludes meaningful statistical analysis.

DATA ANALYSIS/PRESENTATION

Data collected from large studies require careful scrutiny to ensure that data base generated is a correct representation of what transpired during the clinical

Table IV. *Signs and symptom scores frequency distribution at baseline (all subjects in efficacy analyses)*
Scores: 1 = none, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe. Triam = triamcinolone acetonide

Sign or symptom	Treatment	Score 1	2	3	4	5	*N*
Table IVa							
Pruritus	HOE 777	2	0	35	24	5	74
	Vehicle	1	13	27	19	6	68
Erythema	HOE 777	1	17	49	7	0	74
	Vehicle	0	25	32	9	0	68
Scaling	HOE 777	4	27	36	7	0	74
	Vehicle	2	20	39	5	0	68
Thickening	HOE 777	11	17	36	10	0	74
	Vehicle	7	21	31	7	0	68
Lichenification	HOE 777	25	0	29	12	0	74
	Vehicle	21	5	33	7	0	68
Vesiculation	HOE 777	61	10	2	1	0	74
	Vehicle	50	9	7	0	0	68
Oozing	HOE 777	64	15	3	2	0	74
	Vehicle	46	11	6	3	0	68
Crusting	HOE 777	62	14	0	2	0	74
	Vehicle	44	13	7	2	0	68
Table IVb							
Pruritus	HOE 777	1	7	20	25	3	56
	Triam	0	5	20	30	3	58
Erythema	HOE 777	1	6	31	13	5	56
	Triam	0	8	25	24	1	58
Scaling	HOE 777	0	6	27	19	4	56
	Triam	0	3	33	19	3	58
Thickening	HOE 777	1	11	23	17	4	56
	Triam	1	9	20	25	3	58
Lichenification	HOE 777	5	9	24	13	5	56
	Triam	1	8	26	21	2	58
Vesiculation	HOE 777	23	12	15	5	1	56
	Triam	26	14	16	1	1	58
Oozing	HOE 777	30	13	8	4	1	56
	Triam	31	19	6	1	1	58
Crusting	HOE 777	25	15	11	4	1	56
	Triam	29	15	12	1	1	58

trial. The data base also must undergo a certain amount of "cleaning" to define the population to be studied for efficacy. Such cleaning removes patients whose data, for various reasons, might not be a valid representation of the effects of treatment. For example, patients with significant dosing violations should be excluded; data from patient visits exceeding a specified time range should be excluded for the specific visit; data from patients who do not meet protocol entry requirements, used proscribed concurrent medications or were involved in other protocol violations also should be removed. While such "clean-up" rids the data base of interfering variables, it also can introduce a bias. Therefore, results for the "efficacy" population should be compared with those for the entire population and the reasons for any discrepan-

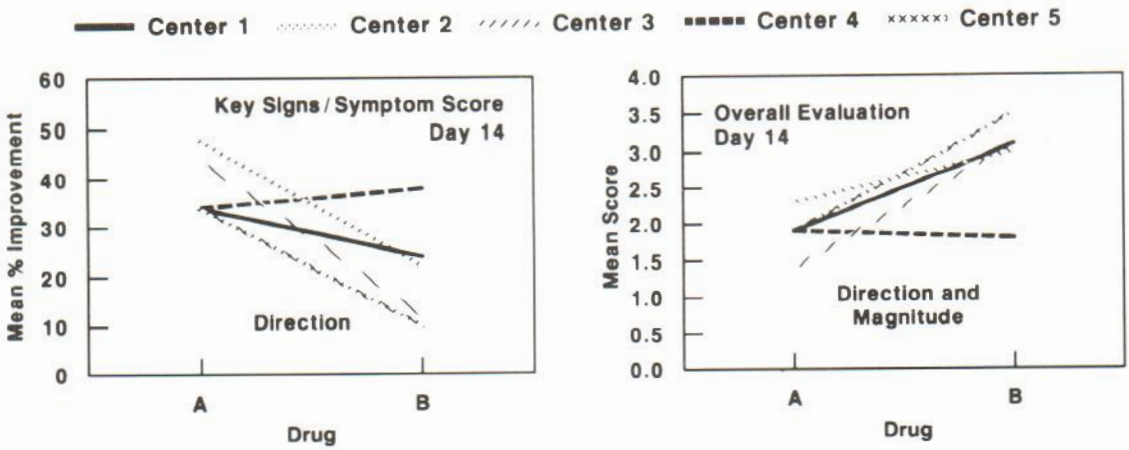

Fig. 1. Results from five centers participating in a multicenter trial comparing two topical corticosteroids in atopic dermatitis. Different lines represent results from different centers. Direction of slopes of lines reflects which treatment (Drug A or B) was better; angles of slopes reflect the magnitude of differences between treatments. (A) Mean percent improvement in key total sign/symptom severity scores after 14 days treatment. Key sign/symptoms (erythema, induration, pruritus) each were graded 1 = none, 2 = slight, 3 = moderate, 4 = severe. (B) Overall evaluation scores: 1 = > 76% clinical improvement; 2 = 51%–75% improvement; 3 = 26%–50% improvement; 4 = ⩽25% improvement; 5 = exacerbation.

cies in outcomes of the two analyses should be investigated. Analyses from the final visit of the efficacy population and the last visit—whenever it occurred —both in the efficacy population and whole population also should be compared as an aid to detect problems which might be causing early termination of patients. Analysis of drug safety data—determination of reasons for dropouts, evaluation of adverse experience reports and analysis of laboratory data—must be performed for the entire patient population, i.e., all patients who received any treatment.

Once the data base is established, a number of analyses can be performed. Obviously, analyses that provide answers to the questions stated in the study objectives should be given primary consideration. The mean change from baseline of sign/symptom severity scores provides a good comparative efficacy measure since comparison of mean scores alone is misleading if baseline scores in the comparative treatment groups are not equal. The severity scores for the key signs/symptoms (erythema, induration, pruritus) can be totaled and the mean change from baseline and mean percent improvement can be calculated to provide an overview of drug efficacy on the major signs and symptoms of disease. Comparison of results of improvement in individual sign or symptom scores and total key sign/symptom scores can be informative for defining the differential activities of an agent. For example, some drugs might be extremely antipruritic, but poor antiinflammatory agents. In such cases, the score for pruritus would reflect dramatic changes; that for the key sign/symptom score would not be as notably affected.

Mean global scores at each visit provide an indication in the change of the overall condition of the patient for all lesions treated. Comparison of these results with those at the target lesion provides an indication of whether the results from target lesion were in fact a valid model for the drug effects on the disease.

While mean values are useful for compressing many results into a single value, they can be misleading since without "qualifiers": they cannot reflect the variability in the measurements made and the range of the values included. Results from three placebo (vehicle) controlled topical corticosteroid clinical studies in atopic dermatitis are presented in Table V (5, 6). In all cases, pooled mean results from all centers participating appear better for the active drug than for the vehicle. However, when one examines the ranges of mean values from the different centers, many of the results for the vehicle and active drug show a great deal of overlap. Because of the deceptive nature of a mean value, comparisons of means should be accompanied by indications of their predicative value such as statistical power statements, standard error values or confidence intervals. Tables exhibiting the distributions of scores also are invaluable for as-

Table V. *Responses to therapy in atopic dermatitis corticosteroid studies. Overlap and range of scores for active drugs and vehicle controls*

Drug	*N*/centers	Day 7	Day 14	Day 21
Poole mean percent improvement in key sign/symptom score 4.6 (range of score at individual centers)				
Active	74 5	45 (31–60)	60 (49–72)	–
Placebo	66 5	25 (6–37)	33 (13–61)	–
Active	89 4	–	–	76 (71–82)
Placebo	90 4	–	–	44 (24–54)
Active	51 3	–	–	84 (72–92)
Placebo	52 3	–	–	23 (7–39)
Global evaluation (1 = none; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe)				
Active	74 5	2.5 (1.0–3.0)	1.9 (1.4–2.3)	–
Placebo	66 5	3.2 (2.8–3.8)	2.9 (1.9–3.6)	–

sessing the full spectrum of results. Such distributions can be complete or grouped. In the former case, using global evaluation scores as an example, one would present numbers of patients with each global improvement score. In the latter case, one could group the scores into clinically meaningful groupings: patients clear vs all other ratings; patients with scores reflecting >50% improvement vs. patients with all other ratings.

Data also must be analyzed to determine whether there were any heterogeneity between the treatment group populations at baseline. The populations' distribution of race, age, sex, and disease severity, duration and state at baseline should be compared as appropriate; the impact of any differences on the study outcome must be determined. Also of importance is an analysis to detect data interactions. In multicenter trials, for example, analysis of variance can be used to determine if results varied significantly at the different study centers involved. Fig. 1 illustrates how results from different study centers can present different pictures of drug efficacy. In this multicenter corticosteroid study in atopic dermatitis, the mean improvement in key sign/symptom scores was better at Day 14 for drug A than for Drug B at four centers; however, at one center, results for drug B were better than those for drug A. Overall evaluation scores showed similar discrepancies between centers. Bias introduced by some investigators (inadvertently, of course) can affect the validity of conclusions from pooled data. The cause or significance of such data interactions must be assessed.

CONCLUSIONS

The basic questions in drug-related clinical research —does the drug work and if so, how does it compare to known standards—often are unanswerable after review of more than one study in the literature. The problem is not necessarily a reflection of poor clinical practice by the investigators. Generally, after careful evaluation, it can be related to such factors as a poor definition of the population studied, variability of the data collected, and/or inappropriate sample sizes studied. Clinicians should be aware of the numerous sources of error and variability in clinical trials when making conclusions from study results, and should insist that clinical trials presented in journals contain adequate information to define the limits of the generalizations/conclusions being made.

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Atopic Dermatitis in the First Six Months of Life

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1 476/2 320 of our cases of atopic dermatitis (AD) start in the first six months of life. The diagnosis is usually easy, but at this age it is sometimes more difficult, mainly because of lack of or rare evidence of scratching, but also because of the brief clinical history that does not allow observation of the characteristic chronic and relapsing course. Moreover, the major atopic disorders—asthma, rhinitis—usually appears later in the natural history of atopic subjects. From a differential diagnosis point of view, AD is the most definite dermatological disorder at this age. Other not well defined conditions occurring in the first six months of life are usually referred to as infantile seborrheic dermatitis, a name that has been used for at least four different disorders: cradle cap, cradle cap with involvement of inguinal, axillary and retroauricular folds, napkin psoriasis and Leiner's erythroderma. From a clinical point of view, AD in the first months of life is characterized by the prevalence of exudating lesions; moreover, the lack of or the rare evidence of scratching allows us to observe isolated vesicular lesions that are found with difficulty in the further course of the disease.

Most cases of atopic dermatitis start in the first six months of life (Table I). The diagnosis of AD is usually easy even though at this age some difficulties arise due to the lack of or rare evidence of scratching and for the brief clinical history that does not allow observation of the characteristic chronic and relapsing course, and moreover, the lack of association with the major atopic disorders, such as asthma and rhinitis, that usually appear later in the natural history of atopic subjects. Asthma occurs in 11% of our cases with atopic dermatitis; in these cases the mean age of onset of asthma is 3.6 years, whereas the mean age of onset of AD is 6 months. This is why asthma occurs in only 5% of our AD patients aged less than 6 months.

From a differential diagnosis point of view, AD is the most definite dermatological disorder in the first six months of life. Other not well etiologically defined conditions occurring at this age are usually referred to as infantile seborrheic dermatitis, a name that has been used for at least four different disorders: cradle cap, cradle cap with involvement of inguinal, axillary and retroauricular folds, napkin psoriasis and Leiner's erythroderma.

The so-called cradle cap is characterized by white-yellowish squamous lesions of the scalp and glabellar region, usually starting in the second month of life and lasting for a period of 4–12 weeks. The differential diagnosis with atopic dermatitis is based on the lack of itching, exudating lesions and involvement of the cheeks. However, about 30% of cases of AD starting in the first months of life, involve, often primarily, the scalp with lesions resembling seborrheic dermatitis. This is why some authors (1) believe that seborrheic dermatitis does not exist and can be considered as a mild type of AD.

We do not know if the frequent mild scaling of the scalp can be considered a very mild AD, but certainly many cases of scaling dermatitis of the scalp, especially when the scalp is extensively involved, are followed by typical atopic dermatitis. In these cases it is often possible to observe exudating lesions under the ear lobes or on the forehead or a mild scaling of the legs.

The second condition which has been included in the spectrum of seborrheic dermatitis, is a disease involving the scalp with squamous lesions and the inguinal, axillary and retroauricular regions with exudating lesions. This condition usually persists for a few months; sometimes it is more persistent and becomes a typical inverted psoriasis, a condition usually associated in children with atopic diathesis.

The third disorder which has been considered by

Table I. *Age of onset of atopic dermatitis in 2 320 cases*

1 month	427	4 years	64
2 months	299	5 years	26
3 months	448	6 years	26
4 months	121	7 years	4
5 months	100	8 years	9
6 months	81	9 years	4
7–12 months	226	10 years	13
1–2 years	332	11 years	4
2–3 years	128	12 years	8

Table II. *Diaper rash and napkin psoriasis. Age of onset*

	Diaper rash	Napkin psoriasis
1 month	65	32
2 months	21	12
3 months	17	7
4 months	9	2
5 months	14	7
6 months	9	5
7–12 months	27	7
1–2 years	12	3
2–3 years	9	2

some authors as seborrheic dermatitis is the so-called napkin psoriasis. The initial lesions are characteristically located in the diaper area and persist in this region for a period of weeks or months. After this period, secondary lesions tend to appear suddenly. They develop first and sometimes exclusively on the face, then on the trunk and limbs. Initially, the secondary lesions are modest in size but with time they spread centrifugally, sometimes completely covering the skin surface. Lasting 4–12 weeks, the lesions disappear and do not reoccur, even when diaper rash relapses.

Some authors include this disorder in the spectrum of seborrheic dermatitis. We do not know exactly what seborrheic dermatitis is, but most authors state that this disorder occurs in the first four months of life, mainly involving the scalp with squamous yellow lesions. On the contrary, diaper rash with secondary eruption occurs at any age in the first two years of life

Table III. *First localization in 2320 cases of AD (%)*

Early = in the first 6 months of life, late = after 6 months

	Early onset	Late onset
Face	79	54
Scalp	12	
Hands	3	12
Groin	2	2.5
Lower limbs	2	6
Trunk, neck	0.5	2
Limb folds	0.5	18
Upper limbs	0.5	1.5
Retroauricular	0.3	3
Generalized		1

Table IV. *Type of lesions in 2320 cases of AD (%)*

	Early[a]	Late[b]
Exudating	49	22
Eryth-squamous	27	28
Erythematous	11	11
Impetiginized	7	1
Vesicular	3	3
Nummular	1	5
Papulo-vesicular	1	7
Infiltrated	1	5
Eczema-like		10
Lichen-like		6
Pompholyx-like		1

[a] In the first 6 months.
[b] After 6 months.

(Table II) and, when the scalp is involved, the lesions, at least initially, are papular in type and isolated.

Napkin psoriasis should be differentiated from generalized atopic dermatitis. AD exceptionally starts in the diaper area. During the course of AD the diaper area is involved in only 20% of cases, in spite of the well-known irritant capacity of urine and feces. In these cases the lesions in the diaper area are usually transient and less erythematous than at other sites. On the other hand, in 80% of subjects with atopic dermatitis, the diaper area is surprisingly spared, even though the entire skin surface is involved.

The fourth condition which has been included in the spectrum of seborrheic dermatitis is Leiner's erythroderma. This condition was more frequent in the past, maybe due to the use of irritants such as tar: in fact we observed 20 cases from 1971 to 1982 and only two cases in the last 6 years. It is usually observed in the first 2 months of life, it lasts 15–30 days and do not reoccur. 10/22 patients with Leiner's erythroderma were revisited after an average period of 8 years. Five children had not had any further skin problem. The other 5 had had minor problems such as minimal AD (4 cases), psoriasis-like eruption (1 case), bullous pyoderma (1 case), vesiculo-papular acrodermatitis (1 case) and papular urticaria (1 case). In one case of Leiner's erythroderma we could observe a previous diaper rash and, subsequently, a secondary eruption with initially isolated erythematous lesions becoming confluent and entirely covering the skin surface in one day. Possibly this condition is the most severe type of napkin psoriasis.

From a clinical point of view, all the sites may be involved in the first six months of life (Table III), the

lesions on the face and scalp usually being the first localization of the disease.

AD in the first six months of life is characterized by the prevalence of exudating lesions; moreover, the lack of or rare evidence of scratching allows us to observe isolated, initial papulo-vesicular lesions that are found with difficulty in the further course of the disease.

Table IV shows that exudating and impetiginized lesions are more frequent in the first 6 months of life, whereas infiltrated, lichen-like and nummular lesions are rare at this age.

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The Management of the Problem Atopic Child in 1988

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Are there really any changes in the management of the problem atopic child in the last twenty to thirty years.

Though there are arguments even among expert dermatologists about the correct method of use of the topical corticosteroids in the atopic child, when one analyses these apparent differences critically and talks to individuals, the differences are more those of the published word than the practical acts. Some authors state categorically that all they use in childhood eczema is 1% hydrocortisone preparations. Analysis of their clinical practice does not, however, support the accuracy of their remarks. My cynical view is that many of us are afraid to put forward our real views for fear that they will be incorrectly interpreted by primary care physicians and that damage to our patients may result. The aggressive and rational use of topical corticosteroids remains the greatest advance in the management of eczema in the last three decades and it is my belief that we should now be educating all those concerned in the management of these children in the correct method of handling potent topical steroids in even young children. The misery caused to patient and family by uncontrolled eczema is far and away more serious than possible steroid side effects. We are all carried away by the anecdotal reports of serious side effects and even death caused by the inappropriate use of large quantities of potent topical steroids in children and, it is salutory to note that the worst case reports have been in psoriasis and not in eczema. There is also little doubt now that topical steroids do not stunt childrens growth unless used in gross excess or in insufficient dosage to control the stunting disease (atopic eczema). Many children cross growth percentile lines after effective control of their eczema, often with quite high potency topical corticosteroids.

It is now widely recognised that the use of gradually reducing concentrations of topical corticosteroids give rise to the longest remissions and the best control. Sudden changes from high to low potency steroids must be avoided at all costs. In my personal view I believe that education of the parents in correct use of varying potency of topical corticosteroids is relatively easy and worthwhile. All my patients are in possession of topical corticosteroids of widely varying potencies with detailed instruction on their correct usage. The use of diary cards and the return of all empty or part empty tubes to be weighed should also be a part of the routine management of the severe patients.

The role of topical and systemic antibiotics is another field which needs to be discussed. Aly in 1981 (1) first suggested that long term Erythromycin was of value in controlling flaring eczema, but many bacteriologists view this with anxiety as there is an obvious risk of the development of resistant organisms. I have recently become interested in this aspect and have developed a so called sandwich treatment. This involves the application of a topical antibacterial followed a few minutes later by a topical corticosteroid and then a layer of an emollient. This technique has been used on 22 patients so far with very gratifying but totally uncontrolled results. Three of the patients so treated had been given systemic corticosteroids by other physicians before we saw them. One child, the first, had spent 18 of the previous 24 months off school, 11 of them in hospital. She has lost no time off school in the last three years, but rapidly deteriorates if the topical antibiotic is stopped. What I completely fail to understand is why this technique works better than the use of a straight topical steroid antibiotic combination. Other workers are, I know, looking more scientifically at the basis for these sort of clinical results and most of us feel that flares of eczema result from superficial staphylococcal colonisation or subclinical infection. The topical antibacterial we have used is pseudomonic acid. There were three reasons for selecting this particular agent. Firstly the fact that it will never be used systemically, secondly its wide spectrum activity against staphylococci, and thirdly the fact that resistant organisms are very rare. Indeed this antibacterial can eradicate multiresistant methycillin resistant staphylococci.

I next turn to the most emotive and controversial aspect of the management of the atopic child, diet. That food allergy or intolerance occurs in atopic children is beyond argument. Many of our patients identify the responsible food either by refusal or obvious

exacerbation of their eczema, but scientific well controlled information is very hard to find. There are well controlled trials of egg and milk free diets in young infants and I believe all will agree that the use of such a diet for a minimum of 8 weeks in an infant with bad atopic eczema is a well worthwhile exercise. The proportion of children who benefit from such therapy varies widely in the literature from 10 to 65%. One problem seems to me to be that when children are placed in a controlled study of dietary measures, there is a very large placebo effect. The proponents of total exclusion/elemental diets are now more wary than in the past, especially after episodes of anaphylaxis following the introduction of new foods. Another of the very curious features is the ability of the older atopic to ingest foods which caused problems at an earlier age with apparently no reaction.

An even greater problem is raised by food additives in the form of colourants and preservatives. Many children apparently improve when additive and colourant free diets are suggested, but again it is very difficult to do controlled studies, and challenges with the suspected materials are often inconclusive and may be dangerous. That there are some children whose eczema improves with such dietary manipulation is undoubted, but as yet there is no accurate way of determining those children who will respond. Scratch tests, RAST tests and IgG4 antibody levels either alone or in combination still do not give a clear indication of the likely antigens.

The role of inhaled allergens has long been recognised in respiratory allergy but has been overlooked or ignored in cutaneous disease. Control of the allergens or the instigation of desensitising regimens have been an essential part of therapy for decades in respiratory allergy. Recent work suggests that this may play a significant and, indeed, a very important part in the management of atopic eczema. It is now certain that heavy exposure to the house dust mite or its P1 antigen may lead to significant flares of the disease. House dust mite is ubiquitous in the developed world and is increasing in incidence in the Third World and even in tented civilisations. Pollens whilst varying in specific type are again worldwide. Hewitt (2) was the first to report that house dust mite and other inhaled antigens might be important in the aetiology of eczema. At about the same time Kleinhams (3) showed a positive relationship between positive scratch tests and positive RASTs to house dust mite in atopic eczematous children, the percentage of positive scratch tests being higher than positive RASTs, but as no mention is made of the level of IgE, it is difficult to interpret some of his data, though other authors have shown a close relationship between positive scratch tests and positive RASTs in children with very high IgE levels. In the early 80's Mitchell (4) amongst others, reawakened interest in the aetiological importance of the house dust mite in atopic eczema. Several workers, using house dust mite P1 antigen, produced positive patch tests on abraded skin of adult atopics. This evidence of type 4 immune reactions to this antigen only occurred in those with positive scratch tests, high levels of IgE and positive RASTs to house dust mite. These workers confirmed by skin biopsy that the cellular infiltrate was suggestive of the type 4 immune response, thus suggesting that some of the flares in eczema might be due to contact with house dust mite and by inference with some other airborne allergens (5). Other workers have found the results difficult to repeat, but this was the first real evidence that house dust mite could be of real importance. Some remain worried by the fact that these results were obtained on abraded skin, but such abrasion occurs in the atopic especially the child who scratches assiduously. Cell mediated immunity to house dust mite was also demonstrated in 11 out of 16 atopic subjects based on increased DNA synthesis after culture of the patients lymphocytes with house dust mite P1 antigen (6). They were unable to show that this reaction was mediated by IgE and others have suggested that mediation may be via IgG4 antibodies. T cells specifically sensitive to house dust mite P1 antigen were found in the majority of patients with Type 1 hypersensitivity to house dust mite P1 antigen.

Constant and persistant re-exposure to house dust mite and other inhaled antigens may lead to failure by the atopic patient or his physician to recognise their importance, thus giving rise to great difficulty in assessing the results of house dust or pollen control methods so often used successfully in respiratory allergy. There are three possible methods of attempting to deal with house dust mite. Avoidance regimens often helpful in respiratory allergy, hyposensitisation, or the removal of the house dust mite from the patient's environment.

In an open study in general practice, Chait et al. (7) reported improvement in the skin of atopic eczema patients with a house dust mite avoidance regimen. In 1985 Zachariae et al. (8) reported on hyposensitisation regimes over several years in adult atopic eczema patients. Whilst they demonstrated a worthwhile de-

crease in symptoms and signs, they felt that the level of reactions made the results generally disappointing. Others are actively studying desensitisation regimens. Rowntree et al. (9) have developed a double antibody antigen binding assay for house dust mite and other inhaled antigens. They believe this to be very specific and to suggest that both type 1 and type 4 immune reactions are responsible for the atopics reaction to airborne antigens. It is thus in recent years that interest has been revitalised in the role of ingested and inhaled allergens in atopic eczema and there seems little doubt that these aspects have been ignored. It seems clear that there is a vital part to be played by house dust mite control measures in atopic eczema patients, especially in childhood. There is good evidence that such measures are of value in respiratory allergy but as yet only anecdotal evidence in eczema, with the exception of the work of Zachariae. With the availability of Natamycin as a method of controlling the food chain of the house dust mite, a new and exciting chapter was opened.

I considered that those children with an early morning flare of their eczema, following bedtime exposure to house dust mite, could be those where this technique would be helpful. We therefore selected children with an early morning flare, high IgE, positive RAST and scratch tests to house dust mite (some with associated respiratory allergy). We monitored response by topical steroid consumption in grammes per week and clinical state as assessed by physician, child and parent. Twelve children have been treated in an open uncontrolled trial.

Three remained unchanged, three showed moderate improvement (of these two had respiratory allergy which was noted to improve) and six showed really significant improvement with reduction of topical steroid usage by over 50%. The study was undertaken over a period of six months.

I believe further work is required to establish the importance of the house dust mite in eczema, but feel very strongly that case selection must be very careful and the technique only used where there is good clinical or immunological suggestion that house dust mite is playing a part.

We now come to what might be described as the experimental therapies, some have been tried and discarded; azathioprine, levamizole, transfer factor, H2 antagonists, phospodiesterase inhibitors, photochemotherapy (we shall hear more of this in a few minutes, its place is clear in adults and now perhaps in childhood). Phototherapy with both UVA and UVB is an exciting new area.

Oral sodium cromoglycate therapy has been the subject of several trials with conflicting results. This is a useful and safe form of therapy that may help some children. The newer steroids with some differential between topical and systemic effects are another exciting development. Then we have to consider the linolenic acid story on which there is as yet conflicting evidence, many trials of gamma linolenic acid producing conflicting reports though Burton believes that overall the effect of gamma linolenic acid is favourable.

Finally, as a clinical scientist I am becoming more and more aware that in dealing with the atopic child with eczema we must try many of the safer new ideas rather than waiting for controlled trials which in this most perplexing disease may give information which clouds the issue for the individual patient. The disease remains as Sulzberger said, one which perplexes both patient and physician alike.

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Criteria for Atopic Dermatitis in a Chinese Population

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Clinical and laboratory findings were collected from 372 Chinese patients with atopic dermatitis. Based on the data and previous study on the criteria, the authors suggest two basic features and six groups of minor features which were categorized by the possible underlying pathogenic factors; genetics, immunology and pharmacophysiology for the diagnosis of atopic dermatitis. ***Key words: Atopic dermatitis; Criteria; China.***

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Atopic dermatitis (AD) is not uncommon in the Chinese, the prevalence of infantile eczema is 16.5%, and AD is 8.3 per thousand in 7–14 year schoolchildren. The features for diagnosis of AD are at present generally based on the guidelines which were proposed by Hanifin and Rajka (1). In order to investigate if the features for the diagnosis also fit patients with AD in the Chinese, we have studied 372 patients with AD, and controls including patients with other skin diseases without atopy, and normals from kindergartens or schools.

MATERIAL AND METHODS

The clinical and laboratory data collected were as follows (2):

Males predominated, male to female ratio was 1.5: 1.

Onset of eruption was in 72.2% of the patients at the age of less than one year and in 91% less than the age of 12 years.

Personal respiratory atopy occurred in 59.1% of the cases. Asthma was present in 52.4%. Allergic rhinitis was obvisouly increased with age. The onset of allergic rhinitis was later than that of asthma.

Family history of atopy including three pedigreed generations was present in 73.1% of the cases.

Eczematous eruption was up to 98% within 2-year-old patients. Lichenification increased with age from 2% to 94% in patients older than 12 years.

Sites of predilection were different at different ages. Forehead (70.7%), ears (68.3%) in addition to cheeks (56.1%) in infants, extensor surfaces of the legs (52.9%) and popliteal fossa (50%) in childhood, and popliteal fossa (52.8%) in adolescents–adults were noticed.

In addition to the major features, some of the minor features such as xerosis, ichthyosis, palmar hyperlinearity, facial pallor, periorbital darkening, hand dermatitis (AD in hand dermatitis group 3.9% vs. population surveyed 0.8%, $p<0.001$), perifollicular accentuation (AD 63.3% vs. nonAD 19.7%, $p<0.001$) were statistically significantly increased in AD. We did not find a significant increase in features like anterior subcapsular cataract, cheilitis, geographic tongue, infraorbital fold, keratoconus, keratosis pilaris, nipple eczema, pityriasis alba, or pompholyx.

The serum IgE was increased significantly, and more prominent in AD with respiratory allergy.

The numbers and function of T cells were markedly lowered, and the number of sm-Ig bearing lymphocytes were increased significantly.

The white dermographism, acetylcholine delayed blanch test and negative histamine test were significantly marked compared with those of the nonatopic individuals.

COMMENT

Recently Svensson (3) listed the significant symptoms and signs of AD. In his group of $p<0.001$, it was 100% compatibility with our significant findings, such as xerosis, serum hyper IgE, personal and family history of atopy. In the groups of $p<0.01$ and $p<0.05$, 60% and 50% of the items were compatible with ours respectively. So the features of AD are similar in both races, the minor differences exist due to genetic and environmental influences. Based on our and previous studies of clinical and laboratory findings on AD, we simplified basic features and categorized minor features by the possible underlying pathogenic associations.

BASIC FEATURES

1. Pruritus, chronic or chronically relapsing dermatitis
   a. Inflammatory eczematous lesions on the face and extensor surfaces of limbs in infants and children
   b. Lichenification of flexural and extensor surfaces of limbs in adolescents and adults.
2. Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis).

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How Doctor's Advice is Followed by Mothers of Atopic Children

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PERSONALITY OF CLINICAL COOPERATORS

Pediatric dermatologists who examine patients and make a diagnosis for them must obtain related information from the persons who accompany them, unless the children are old enough to communicate with the doctors themselves. This is evident in view of the fact that the overwhelming majority of pediatric patients with skin complaints are infants and children (Fig. 1). When only limited information is obtainable from a patient, the pediatric dermatologist must collect as much data as possible from the surrounding persons and select them for use in examining the patient. Such data depend on the informer's personality to a great extent, so that the pediatric dermatologist needs to evaluate his/her personality in an attempt to assess the provided data accurately. Needless to say, the cooperator, who is important in the implementation of medical care, is the patient's mother in most cases. Therefore, pediatric dermatologists must always bear in mind how the mother of a child with a skin complaint will react. Such a plain fact must be emphasized, since the mother will have watched the patient's abnormality developing. Skin changes are basically different in this sense from diseases in other fields that are impossible to observe visually.

One would think that highly pertinent and useful information on the patient's condition could be obtained from his/her mother, who feels highly responsible for him/her in all aspects and continuously watches his/her abnormal condition in full detail, despite her relative inexperience in medical care. However, this is not always the case, and in fact, there are some mothers who provide very inaccurate, or practically demagogic information. For this reason, pediatric dermatologists must judge each individual mother's personality in an attempt to obtain useful information, closest to the actual condition, for use in examination and diagnosis. Mothers who create problems for the pediatric dermatologist are divisible into several types.

1. *Exaggerative type*

This most common type of mother is immediately revealed when she begins to describe the patient's chief complaints. For example, one of the routine complaints made by almost all mothers of this type is "My child has been feeling itchy, without being able to sleep at night, since his/her birth". Nevertheless, the child in front of the pediatric dermatologist may appear well, and naturally, it is necessary to determine the severity of the itching felt by the patient himself/herself. Mothers with this type of personality tend to spread exaggerated information on the results of medical examination.

2. *Traitor type*

The child of a mother of this type is usually already being treated by other physicians. If the child's condition is exacerbated, because the mother has not observed the instructions for using the prescribed ointment (e.g., apply twice a day), she tries to have the patient healed by another physician. The word "traitor" also implies another tendency: When she visits another doctor, she makes, without fail, an unfavorable reference to the details of treatment by the preceding physician. Caution is required in dealing with this type of mother, because she tends to repeat such conduct. The third type of behavior shown by this category is avoidance of active supply of information, probably because the mother intends to compare the present physician with the previous one with respect to medical ability in treatment and diagnosis.

3. *Distrustful type*

The problems posed by this type of mother are well known. In most cases, she has a very deeply rooted doubt about drugs, which escalates into distrusting the physicians who prescribe them. Steroid ointment is the drug about which the largest number of mothers are doubtful or anxious in the pediatric dermatological field in Japan. It is definitely an advantage to have a good knowledge of the possible side effects of this drug, but it is unreasonable if one is so fearful of side

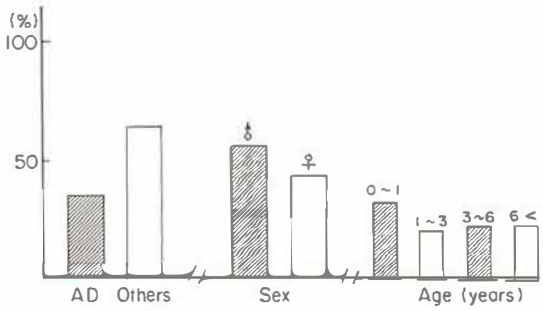

Fig. 1. Details of patients.

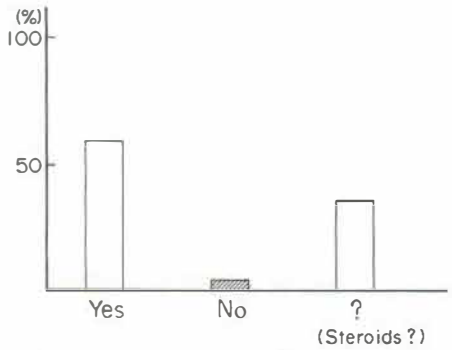

Fig. 3. History of topical steroids.

effects of drugs in general that she distrusts the physician who prescribes the ointment. Most mothers with such a tendency do not use, but store, the drugs prescribed by the physician and instead attempt to treat their child according to a third person's irresponsible advice. Thus the result would be that the patient follows a course unexpected for the doctor.

4. *Specialist type*

In our view, this is a subtype within the distrustful category. Mothers of this type feel that they are competent enough to treat their child's visible skin disease by themselves, rather than leaving the child entirely to the care of physicians who are, in their view, untrustworthy in many respects. Physicians are astonished by their capability of establishing a diagnosis in a dauntless manner without the slightest doubt and taking no notice of differential diagnoses. For example, a specialist-type mother would say conclusively with no detailed explanation or information, "My child has atopic dermatitis". Her sole purpose in bringing the child to an expert doctor is to receive a prescription. If she has to wait long for a prescription, or is received unpleasantly, she would buy drugs of her own prescription from a drugstore to treat her child. It is another characteristic of this type that she does not lose her confidence as a specialist even if the results are not favorable: she blames the drugs for aggravation of her child's condition.

There could be many other types of personality, and some mothers have more than one type. In all cases, the patient is finally treated by his/her mother. Therefore, it is indispensable in the examination and diagnosis of skin diseases in children to grasp promptly the personality of the cooperator in medical care and to come up with the best method for obtaining information from her and for ensuring that the medical instructions are thoroughly observed. Such a strategy is at a level slightly different from medical care or medicine as a science, but should always be borne in mind.

THEIR MANNER IN TREATING CHILDREN

Treatment of atopic dermatitis can become complicated for these various reasons, some medical and some related to quite different matters. These other matters become meaningful issues in routine exami-

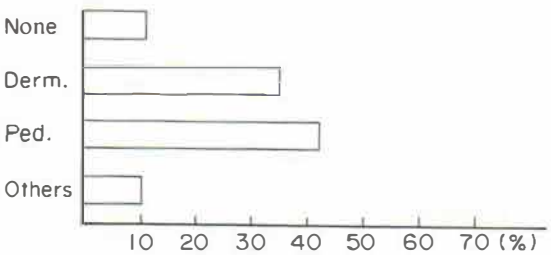

Fig. 2. Physicians who examined the patient before.

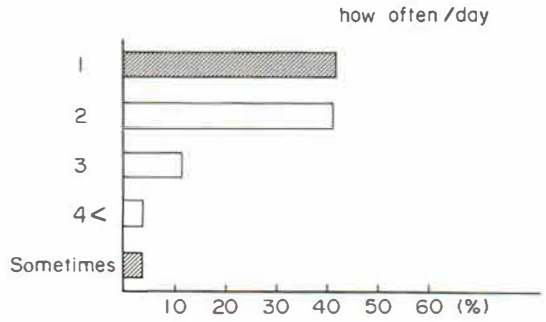

Fig. 4. How topical steroids were used.

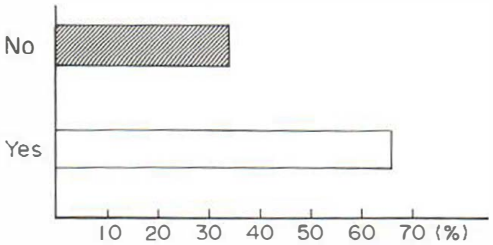

Fig. 5. Did you apply topical steroids honestly according to your doctor's advice?

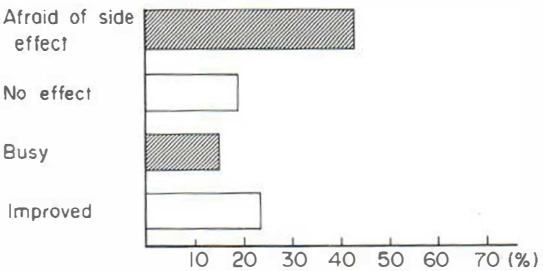

Fig. 6. Reasons not to obey doctor's advice.

nations. We analyzed some of these factors during the period between February and August 1987. Of 771 patients who were examined for the first time during that period, 274 had atopic dermatitis (Fig. 1).

Fig. 2 shows the major specialities of physicians who treated the patients before they visited our outpatient clinic. Only about one-third of the patients were examined by dermatologists, while about one-half were examined by pediatricians, obsteto-gynecologists, surgeons, and so on. This indicates that correct examination for atopic dermatitis is not always performed. Unfortunately, this is the real situation in Japan. So rather cursory treatments are sometimes given by such physicians.

External steroid preparations are used for treatment in most cases (Fig. 3), but in many cases no satisfactory guidance for effective use of these preparations is given. This has caused parents to think of atopic dermatitis as a refractory and serious disease.

When we asked mothers about their actual use of external steroid preparations on the dermal lesions of their children, interesting answers were obtained: 41% or more performed the application only once a day (Fig. 4). In treating atopic dermatitis in children, it is most unlikely that only one application will exert an effect. The resulting insufficient effect of treatment causes parents to hope that various other therapeutic procedures will be attempted for their children.

Even if external therapy exerts no effect, physicians who prescribed the external steroid preparations should have given guidelines for their use. Therefore, we asked parents if they obeyed the guidelines faithfully. One-third of them answered "No" (Fig. 5). In other words, despite the fact that the patients' symptoms did not improve because the parents did not follow the physician's guidance, they abandoned the treatment and changed physicians. There are various reasons for this. For example, the relation between the physician and the parent may be unsatisfactory. Under the present medi-care system in Japan, it is necessary for physicians to examine as many patients as possible. It is believed that as many as 300 patients a day are examined at some private dermatological clinics. Under these conditions parents often have to carry out the treatment of their children according to their own judgment.

We asked mothers why they did not obey the physician's guidelines. Four types of answers were obtained: approximately one-half of the mothers did not cooperate because they feared the side effects of the external steroid preparations (Fig. 6). This indicates a distinct lack of explanation on the part of the physician. Reasons including ineffective treatment and relief of symptoms are recognized to reflect the fear of side effects. On the other hand, about 15% of the mothers said that they were too busy to apply the preparation. However, the mothers seemed to have enough time to apply make-up.

In treating children who suffer from atopic dermatitis, it seems necessary to urge the person who actually carries out the treatment to follow the physician's guidance. If appropriate external therapy and skin care are performed effectively after a good relationship between the patient's mother and physician is established, atopic dermatitis, particularly in children, will become a less troublesome disease.

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Atopic Dermatitis and Atopy in Non-Clinical Populations

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A group of 523 individuals from the general population in the Denver, Colorado area responded to an advertisement for subjects with "problem skin" conditions, such as eczema, dry skin, rashes, etc. The subjects completed screening history information and were evaluated for various characteristics during an examination by a dermatologist. Of the entire population, 33% satisfied the criteria for atopic dermatitis. Another group, described as the generic atopy subset, emerged from the classification and constituted 23% of the total. Individuals in this subset typically had either a personal history of atopic disease or a limited family history. They lacked past or present evidence of flexural rash. A third group, totaling 44%, was classified as non-atopic. A relatively large percent of each of the three groups exhibited some clincial signs of dry skin (e.g., scaling, flakiness). This feature is believed to be due, at least in part, to the damaging effects of the cold, dry conditions in Denver, Colorado. ***Key words: Atopic dermatitis; Atopy; Generic atopy; Non-atopic; Dry skin; Criteria.***

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In the general population, atopy is estimated to occur in 30% of individuals. The prevalence of atopic skin disease is increasing and the incidence is judged to be 10% (1). In cool and/or dry climates, the occurrence is higher than for more tropical environments (2). In most locations in the United States, cool/dry conditions occur at some time during the year. The objective of this work was to select from the general adult population a homogeneous group of individuals with the characteristics of atopic dermatitis to participate in clinical trails in a low humidity setting.

In this paper, the atopic dermatitis criteria and the results of the evaluation of 523 subjects are presented. The features are reviewed for each of the three groups: atopic dermatitis, generic atopy, and non-atopic, which were derived from this population.

METHODS

Subjects

The subjects were volunteers recruited from the general, non-clinical population in the dry climate of Denver, Colorado during the months from July of one year to January of the next. They were contacted via an advertisement for individuals with "problem skin". The specific wording of the advertisement is as follows:

PROBLEM SKIN

If you have skin problems, such as eczema, dry or itchy skin, rashes, or allergies, you could be eligible ... Name of testing agency, phone number

The participants were limited to people between the ages of 18 and 50 years in order to eliminate individuals of elderly xerosis. Both males and females were accepted. The respondents were asked to visit a test facility in Denver to provide a medical history and to be examined by a dermatologist.

Evaluations

At the test facility, the subjects completed a form which provided information about their history of each of the following conditions: recurrent skin disease, eczema, eczema in flexural areas, dry skin, dry skin on hands, family history of skin disease, allergies, hay fever, asthma, family history of allergies/hay fever/asthma, sensitivity to shampoo/laundry detergent/etc. They were then examined by a dermatologist for the current presence or a history of each of the features listed in Table I. The age of onset of recurrent skin disease was also noted.

Criteria

The criteria for classification as atopic dermatitis (AD) was modified from previous schemes (3) for greater simplicity and efficiency of use. To be considered as AD, subjects were required to have each of the following criteria: (*a*) personal or family history of allergy or eczema, (*b*) recurrent eczema (past or present), (*c*) pruritus, and (*d*) rash ever occurring in the flexural areas (elbow creases, popliteals, or behind ears). If any of these four features were missing, at least two of the following modifiers were required: (*a*) onset of skin disease in infancy, (*b*) nipple dermatitis, (*c*) condition worsens with stress or sweating, (*d*) hand eczema in childhood, and (*e*) cheilitis.

For classification as generic atopy, the criteria were the following: (*a*) Two or more of (1) personal or family history of atopic disease, (2) recurrent skin disease, and (3) pruritus; (*b*)

Presented at the 3rd International Symposium on Atopic Dermatitis, Oslo, Norway, in May, 1988.

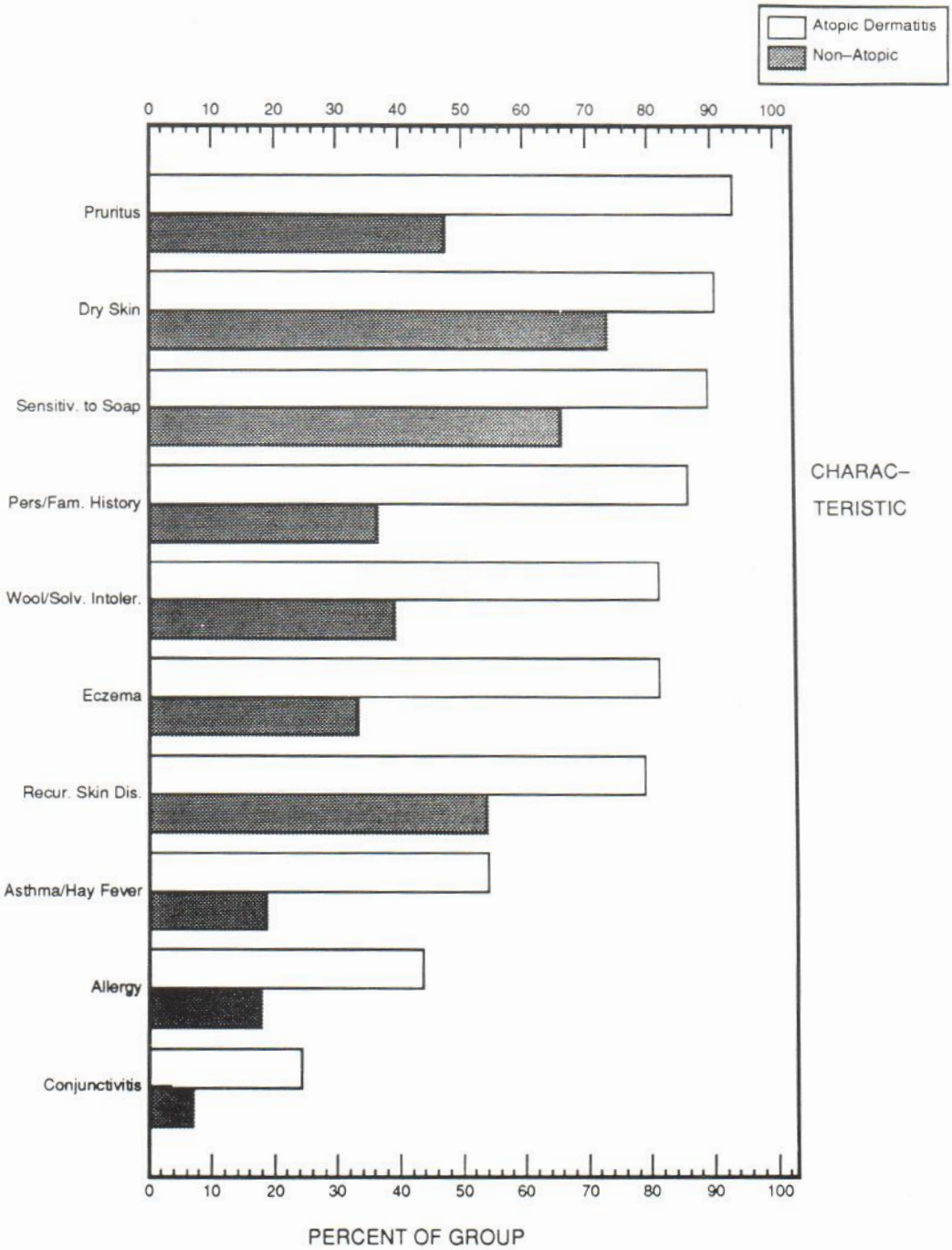
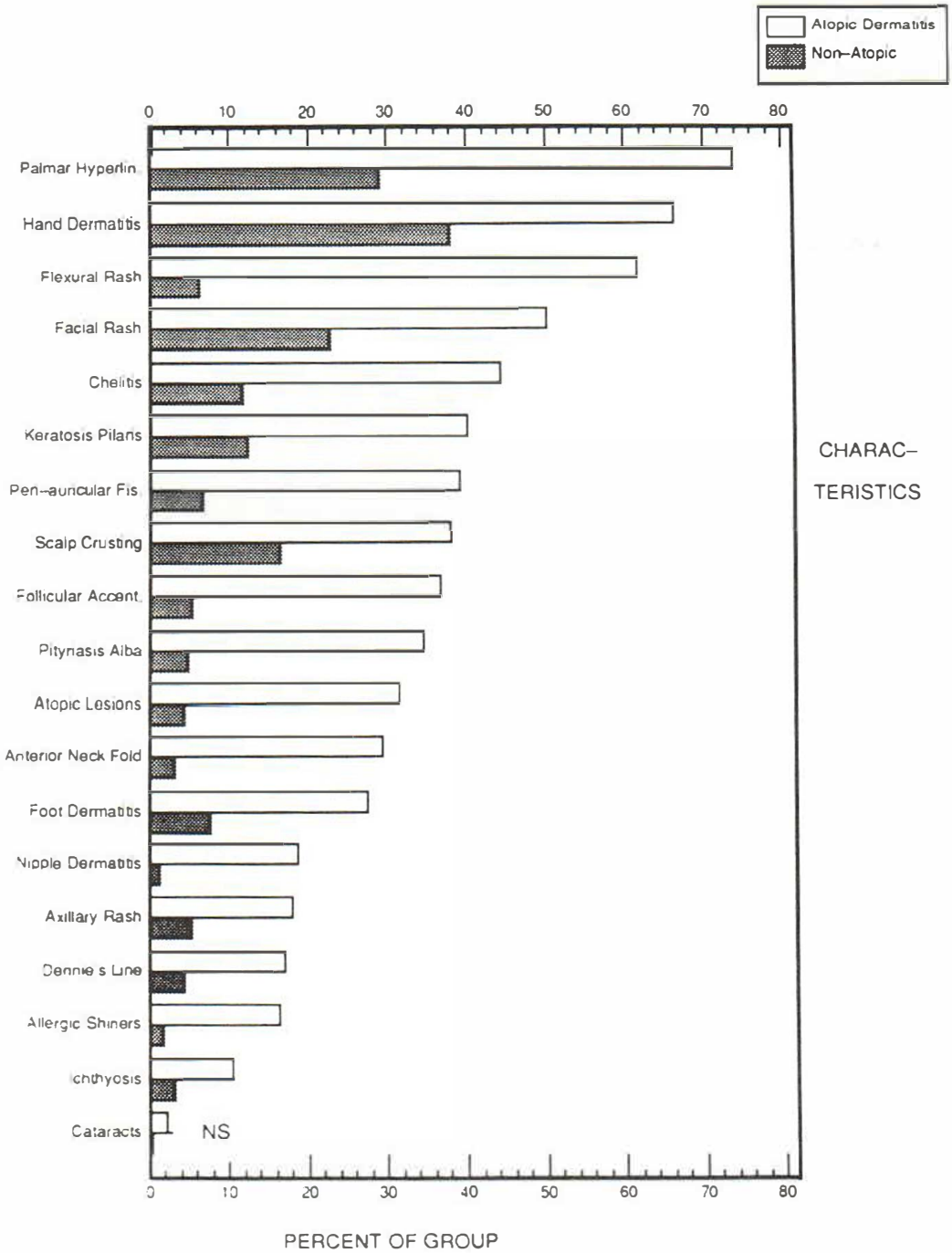


Fig. 1. Characteristics of the atopic dermatitis group compared to the non-atopic group. Each characteristic from the screening history and dermatologist examination is reported as a percent of each group ($n=172$ for the atopic dermatitis group and $n=225$ for the non-atopic group). All differences between the two groups are significant ($p \leqslant 0.05$) unless indicated by NS.



NS indicates differences between the two groups are non–significant.

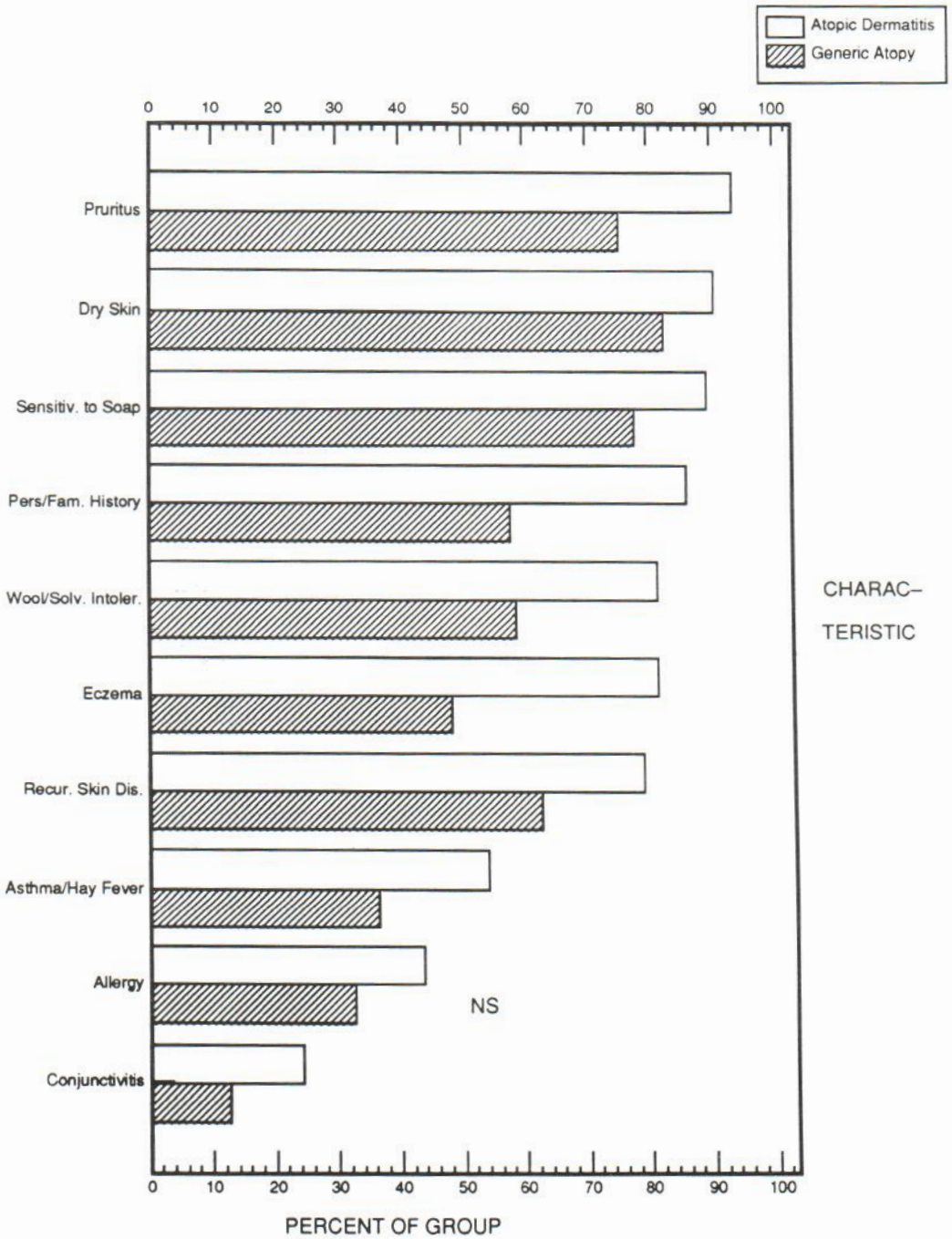


Fig. 2. Characteristics of the atopic dermatitis group compared to the generic atopic group. Each characteristic from the screening history and dermatologist examination is reported as a percent of each group ($n=172$ for the atopic dermatitis group and $n=126$ for the generic atopy group). NS indicates that the differences between the two groups are non-significant. All other differences are signifiant ($p \leqslant 0.05$).

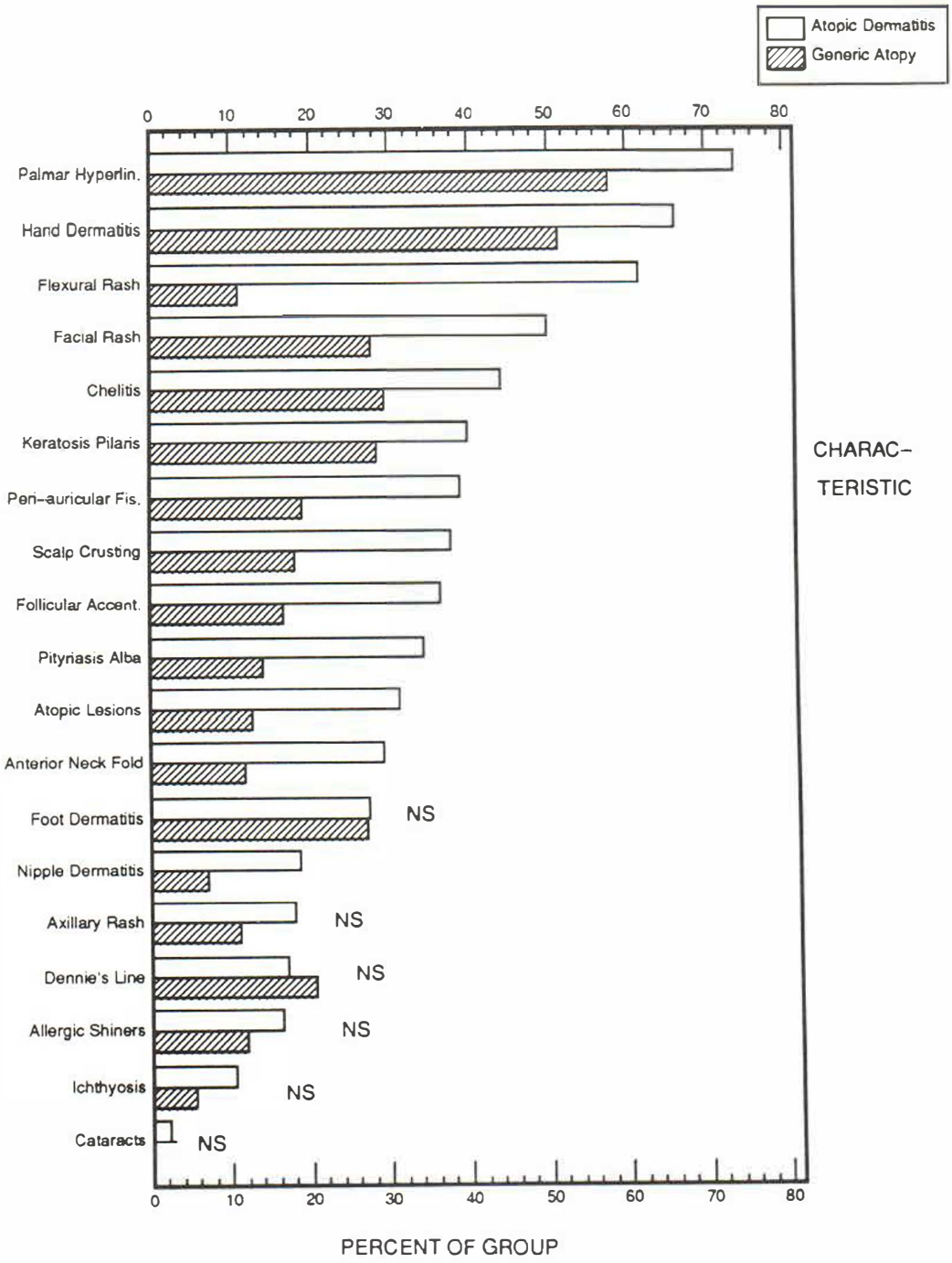

Atopic Dermatitis
Generic Atopy
0 10 20 30 40 50 60 70 80
Palmar Hyperlin.
Hand Dermatitis
Flexural Rash
Facial Rash
Chelitis
Keratosis Pilaris
Peri-auricular Fis.
Scalp Crusting
Follicular Accent.
Pityriasis Alba
Atopic Lesions
Anterior Neck Fold
Foot Dermatitis
Nipple Dermatitis
Axillary Rash
Dennie's Line
Allergic Shiners
Ichthyosis
Cataracts
NS
NS
NS
NS
NS
CHARAC-
TERISTIC
0 10 20 30 40 50 60 70 80
PERCENT OF GROUP

Table 1. *Characteristics of atopic dermatitis group*

History		Physical findings	
Characteristic	Percent	Characteristic	Percent
Pruritus	94	Dry skin	91
Dry skin	98	Palmar hyperlinearity	74
Sensitivity to Products	90	Hand dermatitis	66
Personal/family history of allergy	86	Flexural rash	62
Wool/solvent intolerance	81	Facial rash	50
Eczema	81	Cheilitis	44
Recurrent skin disease	79	Keratosis pilaris	40
Asthma/hay fever	54	Peri-auricular fissures	39
Allergy	44	Scalp crusting	38
Conjunctivitis	24	Follicular accentuations	37
Cataracts	2	Pityriasis alba	34
		Atopic lesions	31
		Anterior neck fold	29
		Foot dermatitis	27
		Nipple dermatitis	19
		Axillary rash	18
		Dennie's line	17
		Allergic shiners	16
		Ichthyosis	10

A family history limited to one parent or sibling; and (*c*) No past or present history of flexural eczema.

Subjects who were classified as non-atopic typically exhibited one of the following: (1) personal or family history of atopic disease, (2) recurrent skin disease, and (3) pruritus.

RESULTS

Over a period of several months, a total of 523 subjects were evaluated. Of these, a total of 172 subjects (33%) were atopic dermatitis (AD). The percentage of individuals exhibiting each of the characteristics from both the screening history and the physical examination is shown in Table 1.

A total of 225 subjects (44%) were classified as non-atopic. A comparison of the atopic dermatitis and non-atopic groups is shown in Fig. 1. Some of the features were present in certain of the non-atopic subjects. However, a statistical comparison of the two groups indicated that there was a significantly higher percentage of AD subjects with each of the characteristics except cataracts. This feature was present in only 11 of the 523 individuals. For the AD group, the most prevalent characteristics were: pruritus, dry skin, personal or family history of allergy/asthma/skin disease, recurrent skin disease, palmar hyperlinearity, wool/solvent intolerance, hand dermatitis, and flexural rash.

The generic atopy group constituted 126 individuals (23% of the total population). A comparison of this group with the AD subset is provided in Fig. 2. For the generic atopy population, the percent of subjects with any given characteristic was significantly lower than the percent for the AD group for all characteristics except: allergy, foot dermatitis, axillary rash, Dennie's line, allergic shiners, ichthyosis, and cataracts. The items which most clearly differentiated these two groups were: flexural rash, eczema, personal/family history of atopic disease (presumably because of the limited family history), facial rash, and wool/solvent intolerance.

For the entire population, the number of subjects who exhibited ichthyosis was 32. This trait was observed in a total of 10% of the AD population.

DISCUSSION

For this non-clinical group of individuals who responded to an advertisement for "problem skin", 33% were classified as atopic dermatitis. Because the advertisement was directed at people with skin problems, this is clearly a selected sample. It is expected that the percentage of AD individuals from the general population at random would be more consistent with the estimated incidence of 10% (1). A second

subset of this non-clinical population, referred to as the generic atopy group, was 23% of the total. These individuals exhibited many of the characteristics of AD. However, for individuals without a personal history of atopy, the family history was generally limited to one parent or sibling (data not shown). The flexural rash was not present upon examination or evident from the history. Together, the two atopic subsets represented 56% of the population, a number which is larger than the estimate of 30% for atopy. This higher incidence is probably due to the fact that a selected group (i.e., individuals with skin problems) responded to the advertisement and were evaluated.

Dry skin (scaling, flaking) was observed by the dermatologist in the physical examination for 91% of the AD group, 82% of the generic atopy subject, and 73% of the non-atopic group. This condition is believed to be due, at least in part, to the damaging effects to the skin of cool, dry climates (4), such as that of Denver, Colorado in the Fall and Winter seasons. It is also noteworthy that a relatively large percentage of subjects in each group report a sensitivity to products such as shampoos, laundry detergents, and wool.

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Short Stature in Children with Atopic Eczema

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Short stature, defined as a standing height below the third centile when corrected for mid-parental height, was found in 22% of children with atopic eczema troublesome enough to cause regular attendance at hospital. The cause of this short stature is unknown in most cases, but contributory factors comprise topical steroid therapy, co-existing asthma, inhaled or oral steroid therapy, malnutrition due to unsupervised dietary restriction, loss of sleep, and vitamin D deficiency. If the short stature is simply associated with severe disease and not attributable to steroid therapy, and if the disease remits before puberty, then catch-up growth can be expected. If the short stature is caused by steroid therapy, or if severe disease persists into adult life, then permanent growth stunting may occur.

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Children with atopic eczema who are referred to hospital usually attend dermatology clinics, and it may be for this reason that the short stature associated with atopic eczema has been seriously neglected. The subject is not mentioned in any of the three major books about atopic eczema (1, 2, 3), all written by dermatologists, and has only recently been the subject of systematic study (4). A number of children with severe eczema now attend paediatric clinics, partly because of the generally handicapping nature of the disorder, and also for the supervision of elimination diets. The result is an increasing interest in the effects that the disease and its treatment have on nutrition (5) and growth.

In the only published study of growth in atopic eczema, 89 children from the Manchester area were examined and nine (10%) had a standing height below the 3rd centile (4). When the height centile was corrected for the mid-parental height, then 22% of the patients were found to be below the 3rd centile. The reduction in height was relatively greater when looking at the sitting height compared with the sitting leg length. The distributions for weight and skinfold thickness were not significantly different when the patients were compared with the normal population. Skeletal maturity was delayed in children with eczema, and this delay was greater in girls than boys. The conclusion was that about one in five children with generalised atopic eczema, severe enough to warrant regular hospital visits, had impaired growth.

The cause of growth impairment in atopic eczema is unknown. There are several possible explanations. It is likely that one or more of these combine in an individual patient to cause short stature. The contributory factors are:

1. *Severe atopic disease*

The Manchester study found that short stature correlated most strongly with the surface area of skin affected by atopic eczema (4). The larger the area affected, the greater was the degree of growth impairment. This finding gives no clue as to whether it is the disease or its treatment which causes short stature.

2. *Topical corticosteroids*

Percutaneous absorption of even the weakest topically applied steroids has been repeatedly demonstrated (6, 7, 8, 9, 10, 11, 12). Adrenocortical suppression (12, 13), dwarfism (14) and iatrogenic Cushing's syndrome (6, 8, 11, 13) are also described following the use of topical steroids. In a recent study of 13 children with atopic or seborrhoeic dermatitis which was being treated with 1% hydrocortisone cream, no less than five were found to have suppressed adrenocortical function as assessed by a 2 hour ACTH test (13). Percutaneous absorption of steroids is enhanced by occlusion (7) and probably by the presence of an inflammatory skin disease such as atopic eczema (15). The site of application influences the rate of absorption, and in one study the absorption of hydrocortisone through the skin of the scrotum was 42 times and the skin of the forehead six times that of the ventral skin of the forearm (16). Nevertheless, despite the use of topical steroids over many years, there are no scientifically based guidelines for a safe weekly dosage of topical steroids in children. Such guidelines as there are for adults (who are no longer at risk of growth impairment) are entirely empirical (17).

The Manchester study showed a progressive relationship between the potency of topical steroids and

short stature (4). This may reflect a direct effect of topical steroids, or it may simply be an indirect effect of disease severity. Six of the 15 shortest patients had been regularly receiving British National Formulary (18) category I or category II (potent or very potent) topical steroids for at least two years. The eczematous lesions in these six patients were not particularly severe or widespread. It is clearly possible that the prolonged use of potent steroids, particularly where there was a large area of skin affected, may well have been a contributory cause of short stature in these six patients. It is desirable to avoid unnecessary prolonged use, over a large skin surface area, of potent topical steroids.

3. *Asthma*

Several investigations (19, 20, 21) have demonstrated an association between asthma and impaired growth, although the cause of this growth impairment is unclear. Asthma may contribute to the short stature seen in children with atopic eczema, and indeed the Manchester study found a relationship between the severity of co-existing asthma and growth impairment (4). Although asthma may be a cause of short stature in atopic eczema, it is also possible that asthma is simply a feature that is correlated with the presence of more extensive atopic eczema. It is theoretically possible that the excessive use of inhaled steroids for the treatment of asthma may contribute to growth impairment, but there was no evidence of this in the Manchester study.

4. *Malabsorption*

Several abnormalities of the gastrointestinal tract have been reported in children with atopic eczema. Defective gastrointestinal handling of certain proteins may play a part in the pathogenesis of atopic eczema, and altered gastrointestinal permeability has been reported by some (22, 23) but not others (24). Furthermore, partial villous atrophy was found among an atypical group of children with eczema (25). Thus malabsorption could in theory contribute to the growth failure seen in atopic eczema, but at present there is no clinical evidence to support this contention. The patients with short stature in the Manchester study did not have loose stools or iron deficiency, and they were not underweight.

5. *Increased nutritional requirements*

Children with severe atopic eczema spend a lot of time scratching, and it is possible that this activity, which can be frenzied at times, and which may continue for much of the night, may increase the child's energy requirements. Heat and protein loss through the skin may also increase nutritional requirements. These aspects have never been investigated, but remain possible contributory causes to short stature.

6. *Unsupervised dietary restriction*

One therapeutic approach to atopic eczema in childhood is to employ an elimination diet (26). Sadly it is not uncommon to see children who have been placed on such a diet without any supervision by a dietitian. Such children are at risk of receiving a nutritionally inadequate diet, and a study in Manchester documented significantly low calcium intakes in children on milk free diets (5). We have also seen patients on very bizarre diets which were deficient not just in calcium but also in protein and carbohydrate, and such children are clearly at risk of growth impairment as well as other nutritional deficiencies (27). Although this aspect has not yet been systematically studied, it is unlikely to be a common cause for the growth impairment seen in children with eczema.

7. *Rickets*

We have seen two children with severe generalised atopic eczema who presented with complete growth failure. Both children were found to have rickets. Their growth improved when they received supplemental calcium and vitamin D by mouth. The cause of the rickets was unclear. The first patient was Caucasian and was receiving a casein hydrolysate milk formula as part of a milk free diet. Unknown to us, she stopped drinking the milk substitute because she disliked the taste, and so her calcium intake became very low. Whether the low calcium intake on its own could have accounted for her rickets was uncertain, and we suspected that a major factor was that she had been excluded from sunlight for about three years.

The second patient was of Asian origin and he too was receiving a casein hydrolysate milk substitute and was very rarely exposed to the sunlight. In his case we again suspected that vitamin D deficiency was the major cause of the rickets, though he may also have been genetically predisposed to rickets because of his Asian origin.

The two lessons here are firstly to bear in mind that the calcium content of milk substitutes is usually considerably less than that of ordinary cows milk (5), and that one has to be on the look out for a child who stops drinking his or her milk substitute. The other

point is that some children with severe eczema are kept out of the sunlight by their parents, and such children are bound to require oral vitamin D supplementation.

8. *Loss of sleep*

Severe atopic eczema invariably interrupts and prevents sleep, and this might interfere with growth hormone release. However in an unpublished study of children with atopic eczema and severe short stature, we found normal growth hormone release during sleep and it is unlikely that failure to release growth hormone is the mechanism of short stature. It is unknown whether prolonged sleep deprivation in atopic eczema could reduce the overall time available for physiological growth hormone release.

9. *Systemic steroids*

A few children with severe generalised atopic eczema unresponsive to conventional therapy may require prolonged treatment with systemic steroids, and clearly such patients are at risk of short stature. If oral steroids are required then it is worthwhile trying to administer them on alternate days, as this may cause less growth impairment (28, 29, 30). If daily steroids are required then to achieve a minimum growth inhibiting effect the steroids should be given as a single dose first thing in the morning (30, 31).

If treatment with steroids is prolonged then catch-up growth may not occur when steroids are stopped (32). It used to be thought that ACTH caused less growth stunting effect than oral steroids (33), but this is now known to be untrue (34).

PROGNOSIS

Without longitudinal studies, the future for children with growth impairment associated with atopic eczema is uncertain. Logic dictates that if the short stature is related to severe disease rather than steroid therapy, and if the disease remits before puberty, then catch-up growth can be expected. This has been our undocumented observation, and it is consistent with observations made in children with asthma (9). If the short stature is caused by steroid therapy, or if severe disease persists into adult life, then permanent growth stunting may occur.

CONCLUSIONS

Short stature, defined as a standing height below the third centile when corrected for mid-parental height, is found in approximately 20% of children with atopic eczema troublesome enough to cause regular attendance at hospital. The cause of this short stature is unknown in most cases, but contributory factors comprise topical steroid therapy, co-existing asthma, inhaled or oral steroid therapy, malnutrition due to unsupervised dietary restriction, loss of sleep, and vitamin D deficiency. It is clearly desirable to avoid unnecessary prolonged use of potent topical steroids especially over a large skin surface area. It is essential that a dietitian supervises any exclusion diet. Children with eczema who avoid the sunlight are likely to need oral vitamin D supplementation.

Severe atopic eczema is often complicated by asthma. The treatment of this, and the assessment of growth and nutrition, remain the province of the paediatrician, and there is a case to be made for paediatricians to take an increasing interest in the management of severe atopic eczema in childhood.

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Immunobiochemical Aspects of Atopic Dermatitis

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A long-term goal is to understand the pathomechanisms of atopic dermatitis. A major advance in the understanding of atopy was provided by observations from bone marrow transplantations which documented the transfer of the atopic diathesis by marrow cells (1). Conversely, the eczema of patients with Wiskott-Aldrich syndrome resolves after bone marrow transplantation (2). Thus, the immune and inflammatory cells that populate and infiltrate the skin in atopic dermatitis, or the nasal membranes in allergic rhinitis, or the bronchial mucosa in asthma, appear to be "vectors" that predispose the tissues to the hyperreactivity typical of the atopic diathesis.

A wide variety of factors have been reported to trigger flares of atopic dermatitis (3) (Table I). Of these, only stress and foods have been documented to cause flaring of dermatitis under controlled, experimental conditions (4, 5). Graham & Wolf reportd increasd skin temperature and decreased reactive hyperemia during experimental emotional stress interviews (4), a phenomenon frequently observed in the clinical setting. The mechanism for these reactions is not fully understood, but it seems likely that the initial event is the release of mediators from skin mast cells. Neuropeptides such as Substance P stimulate histamine release from skin mast cells and may link the central nervous system to cutaneous inflammatory cells (6, 7). Sampson has shown that double-blind food challenges cause itching and erythema accompanied by increased plasma histamine (5, 8) and later infiltration of eosinophils (9). Current concepts suggest that, following mast cell mediator release, infiltrates of basophils, eosinophils, neutrophils, and mononuclear leukocytes may interact to establish a continuing, subacute immune response (10). This may be a hybrid, with components of delayed hypersensitivity (11) and "late phase reactions" which could account for the chronic, indurated, inflammatory condition typical of AD.

In addition to evidence for abnormal inflammatory activity in atopic dermatitis, there are several lines of evidence indicating defects of chemotaxis and cellular immunity. However, our past studies of these abnormalities suggested they were secondary to the dermatitis and normalized rapidly during clinical remissions (12, 13). Abnormalities of IgE are perhaps the most consistent immunological defect in atopic dermatitis. Serum IgE levels are elevated in approximately 80% of patients and correlate roughly with disease severity (14). Cultured mononuclear leukocytes, from patients with elevated serum IgE, produce excessive quantities of IgE during seven to ten day incubations and IgE production appears to be influenced by T cell factors but, as with inflammatory events, the mechanisms of this dysfunction remain to be clarified (15).

In addition to the in vitro IgE overproduction, another very consistent functional leukocyte abnormality in atopic dermatitis is the hyperreleasability of histamine by blood basophils (16–18). We have been interested in the cellular regulatory defects that allow for hyper-IgE production by B lymphocytes as well as the pathomechanism that allows for excessive basophil histamine release. A number of clinical clues, as well as certain in vitro findings, suggest this may relate to abnormal cyclic nucleotide metabolism in atopic dermatitis (19). The blunted cAMP response to catecholamines was initially interpreted as a beta-adrenergic receptor defect but we found no such abnormality in atopic leukocytes (20) and, along with other laboratories, we showed that this cyclic AMP defect was evident whether cells were stimulated with beta-agonists, prostaglandin (PG) or histamine (21).

These findings led us to the demonstration that reduced cAMP levels in stimulated mononuclear leukocytes (MNL) resulted from excessive hydrolysis by cAMP-phosphodiesterase (PDE) rather than inadequate cAMP production (22). This increased PDE activity was present consistently in MNL from pa-

Table I. *Confirmed and putative activators of atopic dermatitis*

Irritants	Immune complexes
Stress	Mites
Foods	Molds
Staphylococci	Yeasts
Viruses	Human dander

tients with active and inactive atopic dermatitis and also from patients with no dermatitis but only allergic respiratory disease. Non-atopic patients with widespread allergic contact dermatitis had normal levels of PDE activity (22).

Functional ramifications of increased leukocyte PDE activity were studied in two systems. The increased basophil histamine-releasability associated with atopic dermatitis showed a striking correlation to increased PDE activity and the abnormal histamine release was consistently reduced to normal levels by in vitro exposure of cells to the PDE inhibitor, RO 20-1724 (17). Likewise, the elevated IgE synthesis by cultured MNL from atopic dermatitis patients correlated with high PDE activity; exposure of the cells to Ro20-1724 for 1 hour, prior to the 10 day cultures, caused a consistent reduction in IgE synthesis (23).

Thus, excessive PDE hydrolysis of cAMP may have a functional role in IgE hyper-production and in basophil/mast cell hyper-releasability of mediators in atopic dermatitis. We have also been interested in abnormalities of other cell systems. We have focused especially on the blood monocyte, which has a particularly high level of PDE activity in atopic dermatitis (24), and is of major interest to our development of specific anti-PDE antibodies. Interestingly, our chromatofocusing studies have shown evidence of distinct PDE enzymes in atopic lymphocytes and monocytes, raising the possibility that different post-translational changes may be acting in the two cell types, or perhaps, in each of the many cell lines originating from bone marrow (25). Understanding these changes may potentially lead to development of a new therapeutic approach for atopy.

It is obvious from our studies that abnormally high PDE activity is present in atopic disease, in cells that are central to immune function. The resulting, inadequate cAMP levels would be expected to cause a permissive, functional hyper-reactivity which is certainly typical of the atopic diathesis. In therapeutic terms, these studies provide a focus for pharmacologic intervention and indeed, studies have shown the effectiveness of a topically applied PDE inhibitor (unpublished placebo-controlled trial). Additionally, our in vitro studies have shown that chronic, oral theophylline administration is ineffective for atopic dermatitis because of tachyphylaxis (26) and, perhaps, because of inadequate delivery of oral drug to the skin, since intravenous theophylline is rapidly effective at relieving the pruritus of atopic dermatitis (27).

A very important question relating to our research is whether increased PDE activity reflects a basic biochemical genetic defect or whether underlying immunological events, possibly of allergic origin, cause elaboration of factors or differences in immune cellular differentiation which in turn generate a secondary rise in PDE activity. These questions are very basic but potentially have enormous medical and socio-occupational value, considering the substantial proportion of the population carrying the atopic diathesis.

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Leukotrienes in Atopic Eczema

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Involved skin in atopic eczema contains elevated levels of the 5-lipoxygenase metabolite of arachidonic acid, leukotriene B_4. In addition, leukocytes of atopic eczema patients produce increased amounts of eicosanoids upon immunological challenge. These facts and the biological effects of eicosanoids suggest their involvement in the pathogenesis of cutaneous inflammation in atopic eczema, and may provide a new target for pharmacological treatment of this disease.

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Inflammatory mediators are assumed to play an important role in the pathophysiology of cutaneous inflammation in atopic eczema. In skin, these molecules are formed predominantly by macrophages and granulocytes; other cell types, however, particularly keratinocytes, also possess the capacity to form inflammatory mediators and cytokines. The biological activities of mediators speak in favour of their involvement in atopic eczema. They cause:

erythema and oedema by influencing the function of cutaneous microvasculature;

inflammatory infiltrate by their chemotactic activity towards leukocytes;

hyperproliferation of epidermis by stimulating cell growth;

regulation of cellular immune phenomena.

Histamine, the classical mediator of inflammation, is present in biopsies from atopic eczema skin in normal concentrations (1). In a case of hyper-IgE-syndrome however, cutaneous histamine levels were maximally elevated. This finding may partly explain the propensity of patients with this disorder to develop skin infections. Despite normal steady state concentrations of histamine in skin, immunological challenge of biopsy pieces led to enhanced histamine release in atopic versus normal subjects. Still, histamine seems not to play a central role in the pathophysiology of atopic eczema, as can already be deduced from the lack of antieczematous properties of antihistamines despite their usefulness as antipruritic drugs.

Therefore, particular interest centers around eicosanoids, which exhibit all biological activities typical of inflammatory mediators. These substances are derivatives of arachidonic acid (eicosatetraenoic acid) which are formed via several enzymatic pathways. The enzyme cyclooxygenase forms the prostaglandins (PG) E_2, F_2 and D_2 as well as prostacyclin and thromboxane. 12-lipoxygenase synthesizes 12-HETE, and the 5-lipoxygenase enzyme forms 5-HETE and the leukotrienes (LT)B_4, C_4 and D_4. Glucocorticosteroids inhibit the formation of all eicosanoids by blocking the activity of the rate limiting enzyme phospholipase A_2, which releases arachidonic acid from cell membrane phospholipids. This action of the most important antiinflammatory drugs in dermatology points to the possible importance of eicosanoids in inflammatory skin diseases. A monograph reviewing the role of eicosanoids in the skin will give a detailed overview about this field (2).

We performed a systematic study analyzing the concentrations of eicosanoids in lesional and uninvolved skin of patients with atopic eczema in comparison with normal controls and patients with psoriasis (3). No elevation of PGE_2 could be measured in either dermatosis. PGD_2, the main product of arachidonic acid formed by mast cells, could not be detected in suction blister fluid in any of the patient groups using two radioimmunoassays. In contrast, in both diseases a selective elevation of the 5-lipoxygenase metabolite LTB_4 was found. This finding does not represent the simple consequence of cutaneous inflammation, because in UV-B-induced dermatitis, there are normal LTB_4 concentrations in the skin (4).

The elevated eicosanoid levels in tissues could be the result of an enhanced "releasability" of inflammatory mediators in atopic subjects. Leukocytes of patients with atopic eczema showed increased production of eicosanoids after immunological stimulation compared to cells from healthy controls (5).

Inflammatory mediators not only are able to explain the inflammatory changes in the skin, but also may account for the disturbed immune regulation in atopy, because histamine, PGE_2 and LTB_4 may cause a negative feedback regulation of the immune response (6).

Eicosanoids could represent potentially useful targets for pharmacological manipulations in atopic eczema. Inhibition of 5-lipoxygenase with or without cyclooxygenase may be one approach. Alternatively, the identification of LTB_4/12-HETE-receptors on epidermal cells (7) could give rise to the development of specific receptor antagonists.

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Evaluation and Relevance of Atopic Basic and Minor Features in Patients with Atopic Dermatitis and in the General Population

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In a prospective computerized study on atopic dermatitis (AD) several basic and minor clinical features in patients with AD ($n=110$) and a sample of the normal population ($n=527$) was studied systematically and analysed statistically with regard to their diagnostic importance. On basis of chi-square values a diagnostic score system was constructed which might help to establish a firm diagnosis of AD in patients with ambiguous cutaneous inflammatory disease. Based on this score system patients with more than 10 points should be considered atopic, patients with 6 to 10 points are suspected to be atopics. An association between serum IgE and the amount of atopic points was found. Seven percent of the normal population sample proved to be obviously atopic, another 19% were suspected to be atopics. ***Key words: Atopic dermatitis; General population; Clinical features; Atopy score; Nickel allergy.***

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For the diagnosis of atopic dermatitis (AD) an array of basic and minor clinical features proposed by Hanifin and Rajka (1) are in common use. However, many of them can be found in normal individuals who never before had skin problems or eczema at the time of examination. In recent years atopic dermatitis seems to have become more frequent in the general population (2, 3, 4), yet there are only a few studies about the incidence rates of atopy in recent decades. The present study focuses on the evaluation and quantification of anamnestical and clinical features of atopy in clearly established cases of atopic dermatitis (AD) compared to a general German normal population (NP).

MATERIAL AND METHODS

Patients with atopic dermatitis (AD)

Patients with atopic dermatitis (AD: $n=110$; 64 females, 46 males; median age 21 years) were collected from the in- and out-patient divisions of our Department of Dermatology where a special atopy service has been instituted. Patients with all degrees of severity of the disease were seen. The diagnosis of AD was established according to Hanifin & Rajka (1). All patients revealed clinical or anamnestic data of recurrent flexural itching and lichenified eczema. The referral area was both urban and rural.

German normal population (NP)

A sample of the German normal population (NP; $n=554$) were taken from urban and rural areas. Persons with a history or the clinical picture of flexural eczema ($n=27$) were excluded. Thus the sample size of the controls was $n=527$ (178 females, 349 males) largely similar in age (median 23 years) and occupational distribution.

Clinical examinations and laboratory investigations

All anamnestical and atopic basic and minor clinical features described in the literature were examined in both groups. To achieve a good interobserver agreement (5) the clinical examination was performed by two dermatologists simultaneously. Serum IgE and Phadiatop (a RAST for screening inhalant allergy; "Pharmacia GmbH") were investigated in all test subjects.

Statistics

The chi-square test was used to analyse cross-classified data, nonparametric tests (Mann-Whitney rank-sum test and Kruskal-Wallis statistic) to analyse interval and ordinal scaled data (6). The level of significance chosen was $p<0.01$. A score system was constructed based on chi-square values. Relative risks were calculated according to Breslow & Day (7).

RESULTS

Atopic basic and minor features

Anamnestic data as well as clinical findings of atopic basic features in AD and NP are presented in Fig. 1 *a*, of minor features (arranged according to their frequencies in AD) in Fig. 1 *b*. Only those features are seen which had significantly higher incidences and were more frequent than 20% in AD. The frequencies, chi-square values and relative risks of atopic basic and minor features are listed in Table I. The term relative risk (RR) indicates how many times more frequent the disease is in the individuals positive for the atopic feature than in individuals negative for the atopic feature ($p<0.01$).

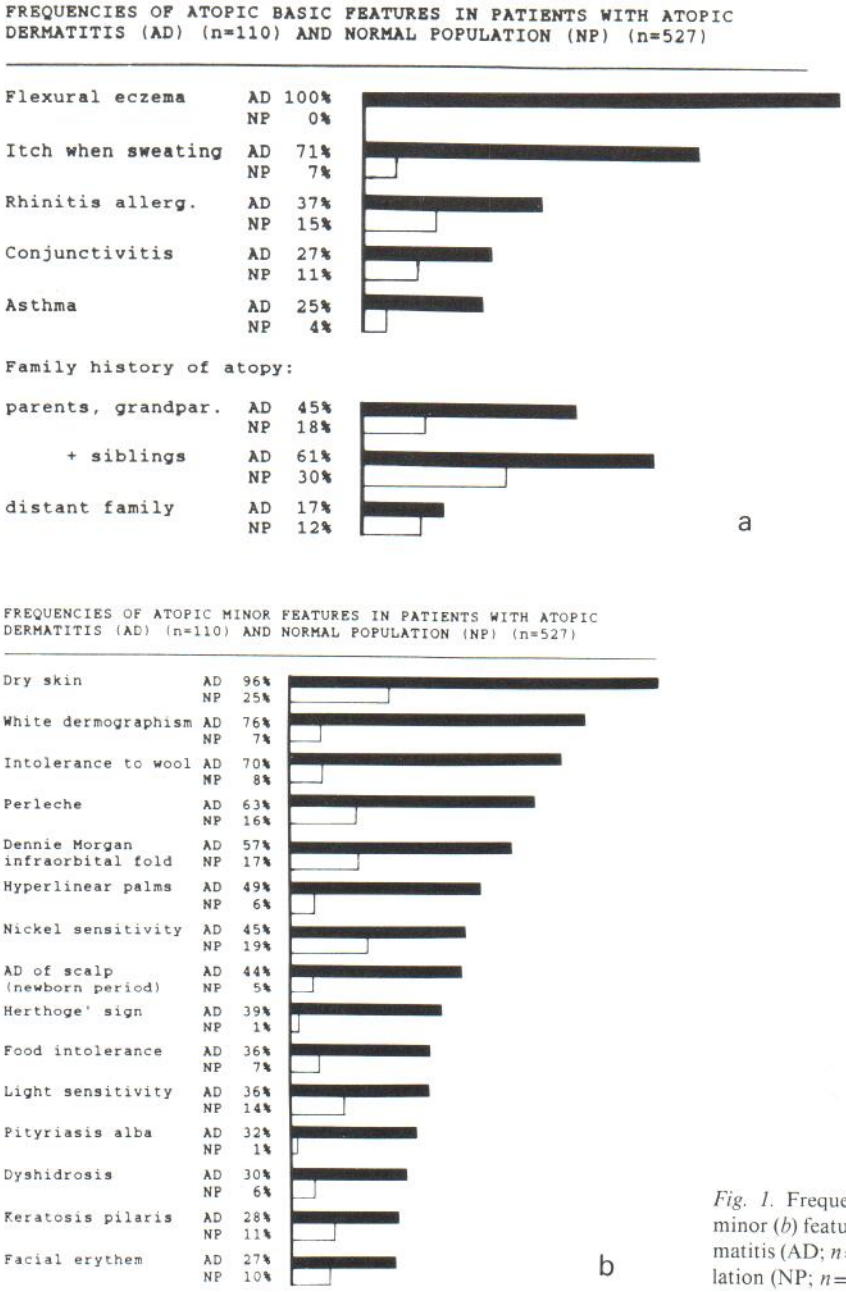

Fig. 1. Frequencies of atopic basic (*a*) and minor (*b*) features in patients with atopic dermatitis (AD; $n=110$) and in the normal population (NP; $n=527$).

The frequencies of a positive family history of atopy was dependent on the included relatives. An immediate positive history of atopy, i.e. of the parents or grandparents was found in 45% (AD) respectively 18% (NP), if the siblings were included as well the frequencies increased to 61% (AD) respectively 30% (NP). There were no statistically significant differences in the distant family history of atopy between AD (17%) and NP (12%) (Table I).

The frequencies, chi-square values and relative risk of some minor features were higher than the values found in basic features. Especially in dry skin, Hertoghe' sign and white dermographism the estimated relative risks were high (Table I). Comparing the fre-

Table I. *Frequencies, chi-square values (χ^2), relative risk[a] (RR) of atopic basic and minor features in atopic dermatitis (AD; n=110) and in the normal population (NP; n=527)*

Atopic features	AD (%)	NP (%)	χ^2	RR[a]
Basic features				
Itch when sweating	71	7	240	33.6
Rhinitis allerg.	37	15	27	3.2
Conjunctivitis allerg.	27	11	19	3.0
Asthma	25	4	57	8.1
Family history of atopy				
parents, grandparents	45	18	39	3.8
parents, grandparents, siblings	61	30	36	3.8
Distant family	17	12	NS	–
Minor features				
Dry skin	96	25	196	78.0
White dermographism	76	7	262	39.6
Intolerance to wool	70	8	222	25.7
Perleche	63	16	105	8.7
Dennie-Morgan's fold	57	17	78	6.6
Hyperlinear palms	49	6	140	15.1
Hist. of nickel sens.	45	19	32	3.4
AD of scalp	44	5	118	13.9
Hertoghe's sign	39	1	173	46.4
Light sensitivity	36	14	29	3.5
Pityriasis alba	32	1	137	35.0
Dyshidrosis	30	6	53	6.3
Keratosis pilaris	28	11	22	3.2
Facial erythem[a]	27	10	22	3.2

[a] The term relative risk (RR) indicates how many times more frequent the disease is in the individuals positive for the atopic feature than in individuals negative for the atopic feature ($p<0.01$).

quencies of the atopic features between females and males there were no significant differences except for the anamnestic sensitivity to nickel, which was highly correlated with females and ear-pierced. Specific features, like atopic winter feet (AD 16%; NP 0%), dirty neck (AD 10%; NP 0%) and nipple eczema (AD 9%; NP 0%) were not listed because their incidences were below 20%. Several minor features have been found worthless because they did not differ significantly: pronounced local insect reaction, herpes labialis, drug intolerance and urticaria.

IgE and Phadiatop were statistically associated with atopic dermatitis ($p<0.001$). There was elevated serum IgE (IgE > 100 U/ml) in 75% and a positive Phadiatop in 68% in AD. Especially in AD both parameters were statistically significant associated with rhinitis and conjunctivitis ($p<0.001$).

Table II. *Score system based on χ^2-values*

3 Points ($\chi^2>150$)
Itch when sweating
Intolerance to wool
Xerosis
White dermographism
Hertoghe's sign
2 Points ($100<\chi^2<150$)
AD of scalp (newborn)
Perleche, cheilitis
Hyperlinear palms
Pityriasis alba
1 Point ($\chi^2<100$)
Family history of atopy
Rhinitis
Conjunctivitis
Asthma
Dyshidrosis
Dennie Morgan fold
Nickel sensitivity
Food intolerance
Facial erythema
Light sensitivity
Keratosis pilaris

Score system

An atopic score system should be based on anamnestic and clinical features without laboratory investigations. Thus serum IgE and Phadiatop were not taken into consideration. The score system was based on statistical evaluation and should be restricted to the frequent important criteria. Specific features were excluded which were less frequent than 20% in AD (atopic winter feet, nipple eczema, dirty neck). The presence of an itching flexural dermatitis was not included since this was the selection basis. On the basis of chi-square values every atopic feature obtained a value between 1 and 3 points according to its statistical significance (Table II). By using the proposed score system both groups were separated fairly well with minimal overlapping (Fig. 2). The summarized atopic points were normally distributed in AD. There were different degrees of atopic severity based on the amount of atopic points. The median of serum IgE in the different groups classified by atopic points (Fig. 3) were significantly lower in NP than in AD

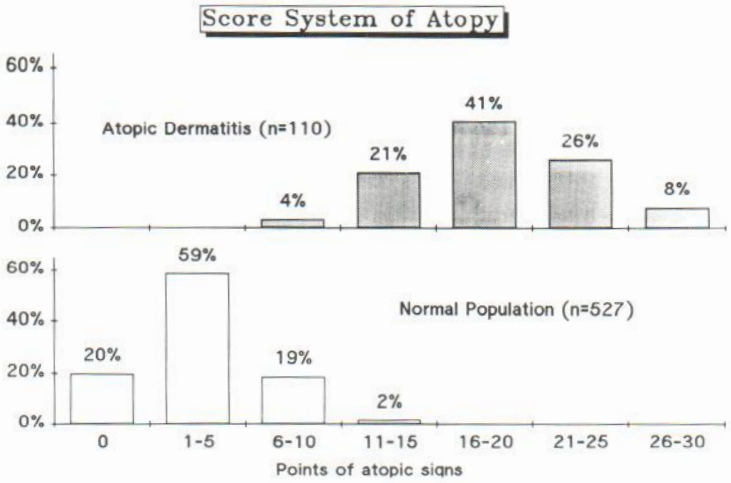

Fig. 2. Frequencies of classified atopic points in atopic dermatitis (AD; $n=110$) and in the normal population (NP; $n=527$). The score system is based on statistical evaluation of atopic basic and minor features according to chi-square values.

(Mann-Whitney rank sum test, $p<0.001$). In the different groups of AD classified by our score system the serum IgE increased significantly (Kruskal-Wallis test, $p<0.01$), but not in NP.

DISCUSSION

The diagnostic criteria for atopic dermatitis proposed by Hanifin & Rajka (1) are based on traditional clinical experience. There was an incomplete agreement between the proposed criteria and the results of some other studies (8, 9, 10). In the present prospective standardized study the occurrence of different atopic features have been compared to those in the general population because the proposed score system is based on relative risks which depend on the frequencies of atopic symptoms and signs in the general population. Some typical basic features were found to be of minor importance because of their high frequency in control material. For example a history of atopic disease in the family is often obtained. Dependent on the number of included family members the frequencies ranged between 45 and 61% in AD and between 18% and 30% in NP (Table I). Frequencies between 43 and 73% were obtained by other studies (8, 10, 11, 12, 13). Most of the former studies did not investigate the frequencies of a positive family history in the normal population simultaneously. Kjellman (13) studied the incidence of atopic disease in a sample of children aged 7 years and its relation to the family history. In our study the relative risk of a history of atopic disease in the immediate family was only 3.8 because of the high incidence in the normal population. A distant family history of atopy was found to be of no diagnostic relevance. Other minor features have emerged as important factors because of their high relative risks (Table II). The frequency of intolerance to wool in AD (Table I; 70%) is in good accordance with Svensson et al. (8) who in 72% found irritations from textiles in AD and in 36% in non-eczematous out-patients. In our general population of young adults only 8% reported an intolerance to wool.

According to Hanifin & Rajka (1) an intolerance to metals is not a diagnostic feature of atopic dermatitis. Romaguera et al. (14) found an atopic history in 49% of 627 patients who complained of intolerance to

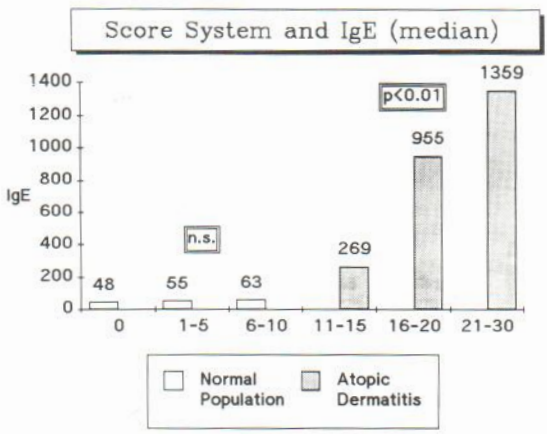

Fig. 3. Median of serum IgE in different groups of the normal population (NP; $n=527$) and atopic dermatitis (AD; $n=110$) classified according to atopic score system which is based on statistical evaluation of atopic basic and minor features according to chi-square values. (Differences of serum IgE between the three groups of AD resp. NP are calculated by Kruskal-Wallis test.)

metals and had a positive patch test to nickel sulfate. In our former prospective study (15) where we investigated the occurrence of delayed-type hypersensitivity in a sample ($n=143$) of patients with AD and comparing them to control subjects similar in age and occupational distribution, atopics were found to have a significantly higher incidence of reactions to nickel. Thus sensitivity to nickel may be regarded as a further minor feature. Because of the frequency in the normal population the relative risk was only 3.4. Sensitivity to nickel can therefore not be regarded as a hard criterion for the diagnosis of AD.

The purpose of the score system was to summarize atopic features in a way that best discriminates atopic risk on the basis of frequent clinical signs without laboratory investigations. Serum IgE, Phadiatop and some specific signs of atopy which were less frequent than 20% were therefore not taken into consideration. In AD the serum IgE were associated with the different groups of AD classified by the proposed score system (Fig. 3). The individual atopic symptom and signs were elevated with regard to their diagnostic importance. The study emphasis the presence of subgroups of atopic dermatitis (Fig. 2). This score system based on traditional atopic features may also be a tool to estimate the atopic risk in non affected individuals. Based on the score system a patient with more than 10 points may be considered atopic, a patient with 6 to 10 points is suspected to be an atopic. Because of a history or clinical manifestation of flexural eczema 5% of the normal population were excluded. Using the proposed score system (Fig. 2) in our sample of the general population another 2% may be considered atopic, another 19% were suspected to be atopics (calculated atopic points: 6 to 10 points). In conclusion the prevalence of atopy in the investigated Caucasian normal population is estimated to be more than 7% and an additional 19% are suspected to be atopics.

ACKNOWLEDGEMENTS

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Langerhans Cells and Atopic Dermatitis

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The epidermal Langerhans cells are situated mostly suprabasally with long dendrites between the keratinocytes. They comprise about 2–3% of the cells in normal epidermis and can be visualized by means of antibodies directed against CD 1 or HLA-DR in immunological staining techniques.

Research in recent years has demonstrated that epidermal Langerhans cells play a major role in the immune reactions in the skin. Their main functions are uptake, processing and presentation of various types of antigens to T lymphocytes (1), and production of the immunoregulatory substance Interleukin 1 functionally similar if not identical to epidermal cell derived thymocyte activating factor, ETAF, which was first shown to be produced by keratinocytes (2).

Using a suction blister technique to obtain epidermal cells we are able to produce epidermal cell suspensions with a viability of usually more than 90%. Using these cell suspensions in functional studies, i.e. coculturing them with allogeneic T lymphocytes or autologous T lymphocytes plus antigen, we have demonstrated that epidermal cells are capable of alloactivation and that they are able to present bacterial antigens like purified protein derivative of tuberculin, live herpes simplex virus and virus antigen, candida antigen, trichophytin and the contact allergen nickel sulphate to T lymphocytes, and thereby induce an antigen specific T-cell response to these antigens in previously sensitized individuals (3–6). Preincubation of the epidermal cell suspensions with a rabbit anti-DR antiserum plus complement abrogated the responses. In the studies with nickel sulphate even preincubation with only the rabbit anti-DR antiserum alone without complement was sufficent to abrogate the responses (6), indicating that the epidermal Langerhans cells were mainly responsible for the induction of the T-cell responses.

The results of these experiments may be regarded as the in vitro equivalent of the afferent phase of the T-cell dependent delayed type hypersensitivity reaction to these antigens in the skin. Furthermore, the induction of the T-cell responses is dependent on the HLA class II DR antigens on the Langerhans cell-surface, blocking of the HLA-DR antigens abrogated the T-cell response. We have also shown that the DR antigens function as restriction elements, that is the Langerhans cells and the T-cells have to share the same DR determinants to be able to cooperate (7). By means of radioimmunoassay technique we have demonstrated that Langerhans cells express more HLA-DR than peripheral blood macrophages and the antigen presenting dendritic cells (8). A per cell comparison between Langerhans cells and peripheral blood macrophages demonstrate that Langerhans cells are much more efficient in inducing a T-cell response to nickelsulphate, indicating that they are highly specialized in antigen presentation (9).

ATOPIC DERMATITIS

Atopic dermatitis is an inflammatory skin disease with pruritus and lichenification of the skin. The patients usually have a personal or family history of allergic disease and demonstrate abnormalities in various immune functions. They have increased susceptibility to cutaneous dissemination of certain viral infections such as herpes simplex and vaccinia (10), and decreased delayed type hypersensitivity responses to common microbial antigens (11). Furthermore they have increased IgE production (12), low incidence of sensitization to contact allergens (13), decreased lymphocyte responses to mitogens and antigens (11) and defective granulocyte and monocyte chemotaxis (14).

The histopathological findings in atopic dermatitis are nonspecific. There is infiltration of mononuclear cells in the epidermis and superficial dermis together with epidermal edema in early lesions and acanthosis in chronic lesions. Increased mast cell numbers and changes in dermal nerves and vessels have also been reported (15).

These histopathological features in the clinically affected skin of patients with atopic dermatitis show similarity to those found in contact dermatitis, and eczematous changes can be induced by specific allergens such as dust mite antigen patch tests (16). The

infiltrating cells in the skin of atopic dermatitis are mainly T lymphocytes (17), with a large majority of helper T-cells and few suppressor T-cells (18). HLA-DR have been demonstrated on most of the infiltrating cells (19), and double labelling experiments demonstrated Interleukin 2 receptor positive T-helper cells indicating functional activation (20). Using the L-dopa histofluorescence technique chronic lesions demonstrate significantly increased number of Langerhans cells throughout acanthotic epidermis, with occasionally focal accumulation (21), while acute erythematous lesions do not demonstrate any change in the number of Langerhans cells in the epidermal lesions (21). Similar observations have been published by others using monoclonal antibodies directed against the T-6 antigen (19).

OKT-6 positive dendritic cells are scattered through the mid and upper dermal infiltrate, with frequent focal clumping and close proximity to T-helper cells and they number approximately 10% of the total infiltrate (22). The overwhelming majority of these dermal OKT 6 positive cells also express HLA-DR as evidenced by double staining experiments (18), and are therefore highly likely Langerhans cells. In contrast to other chronic skin conditions including contact dermatitis, which show HLA-DR positive keratinocytes, the keratinocytes in atopic dermatitis are almost completely HLA-DR negative (19).

Recently the presence of IgE molecules on epidermal Langerhans cells was demonstrated in patients with atopic dermatitis using the indirect immunoperoxidase technique (23). The phenomenon seemed to be specific for atopic dermatitis since skin sections from non-atopic controls and patients with allergic asthma and contact dermatitis did not show epidermal anti-IgE staining. The same authors also found positive dermal anti-IgE staining. Others have demonstrated coating with IgE on T lymphocytes in dermis, sometimes in conjunction with Langerhans cells possibly demonstrating the cytophilic quality of IgE (19). Using a double labelling immunofluorescence technique we have demonstrated a heterogeneous epidermal Langerhans cell population in the skin of patients with atopic dermatitis, about two thirds of the epidermal Langerhans cells carry surface IgE (24). An attempt to demonstrate birch allergen on the surface of the IgE-positive Langerhans cells failed, also after preincubation of the Langerhans cells with a high concentration of birch antigen. Furthermore, the number of IgE-positive Langerhans cells did not increase after 90 min incubation with a serum pool containing a high IgE concentration.

CONCLUSION

In the skin of patients with atopic dermatitis a mixture of type I and type IV reactions is seen, and the same antigen can induce both type I and type IV hypersensitivity reactions. The skin infiltrate consists mainly of activated T-helper cells together with a substantial number of Langerhans cells, as seen in cell mediated immune reactions. Taken together these findings may indicate an antigen-presenting function of Langerhans cells in a T-cell mediated immune mechanism, be it with inhalant, food or other antigens, as part of the pathogenesis of atopic dermatitis.

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Acta Derm Venereol (Stockh) 1989; Suppl 144: 58–63

New Aspects in the Pathogenesis of Atopic Dermatitis

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Atopic dermatitis (AD) has a complex pathogenesis. Many factors may be involved in the circulation and in the skin. In the circulation the most important changes are an abnormal T-lymphocyte function (1) and an increased releasability of basophils (2), which are possibly due to an increased intracellular c-AMP phosphodiesterase activity (3), an increased serum IgE level with a specificity to a wide variety of allergens (4) and blood eosinophilia (5).

In the skin of patients with AD there exists an infiltration of activated T-lymphocytes (with an increased T4/T8 ratio) (6) and antigen presenting cells (CD1+ and RFD1+) (7, 8), lying in the upper part of the dermis and around blood vessels. Although intact eosinophils are only occasionally observed, they may play a role in the inflammatory mechanism since abundant depositions of extracellularly lying eosinophil derived proteins (major basic protein) have been reported in AD skin (9). The increased serum IgE level is reflected by an increased binding of IgE molecules to mast cells (10) and also by binding of IgE molecules to dendritic cells (CD1+) in the epidermis and dermis (11, 12, 13, 14, 15) (Fig. 1). The latter phenomenon is present in clinically involved and, to a lower degree, also in clinically "normal" looking skin from AD patients with elevated serum IgE levels. Immunoelectron microscopy studies on epidermal cell suspensions from AD patients revealed that IgE molecules were present on CD1+ cls containing Birbeck granules and, therefore, being Langerhans cells (LC) (11). Occasionally IgE+/CD1+ cells without Birbeck granules (indeterminate cells) were also observed.

The epidermal anti-IgE staining in AD patients disappears after 2 weeks of local corticosteroid (triamcinolon acetonide) treatment, whereas the epidermal CD1 staining is still present (personal observation).

Since the tissue IgE level is proportional to the serum IgE level it may be expected that the presence of IgE on epidermal LC is not specific for AD and may be observed in other skin diseases with elevated serum IgE levels. Indeed, the clinically involved skin of patients with mycosis fungoides and psoriasis with elevated serum IgE levels may also show a dendritic anti-IgE staining (personal observations).

Further studies on LC enriched epidermal cell suspensions from AD patients revealed that IgE is bound to LC by a Fc-receptor. This FcεR on LC is trypsin resistant, has affinity for IgG, binds with BB10, a monoclonal antibody directed against the FcεR on eosinophils, platelets and macrophages, does not bind to anti-CD23 antibodies directed against the FcεR on B-lymphocytes, and is associated with the CD1 antigen (16). The significance of this latter association is not yet clear.

The question arises whether IgE molecules, present on epidermal LC, have biological significance. *Are IgE molecules on epidermal Langerhans cells specific for and do they bind allergens?*

Patch test reactions to allergens

Environmental allergens (airborne or acroallergens) may reach the skin via the circulation after inhalation or via direct contact with the skin. It is still obscure if eczematous skin lesions in AD patients can be induced after inhalation of allergens (17, 18). Evidence has been presented that aeroallergens can penetrate the skin after direct epidermal contact and induce eczematous skin lesions in AD patients (19, 20). The penetration through the epidermis of molecules with a large molecular weight (compared to classical contact allergens) may be explained by an epidermal barrier dysfunction, which has been described in clinically involved and clinically normal looking AD skin (21, 22, 23). Several groups (20, 24, 25, 26, 27) have reported the presence of delayed type patch test reactions (positive after 24–48 h) to aeroallergens in patients with AD. These patch test reactions may be observed after epicutaneous application of aeroallergens on slightly abraded (24), stripped (25, 27) or even intact skin (26). Furthermore, these delayed patch test reactions with aeroallergens seem to be specific for AD patients, since they cannot be observed in non-atopic normals or atopics without AD.

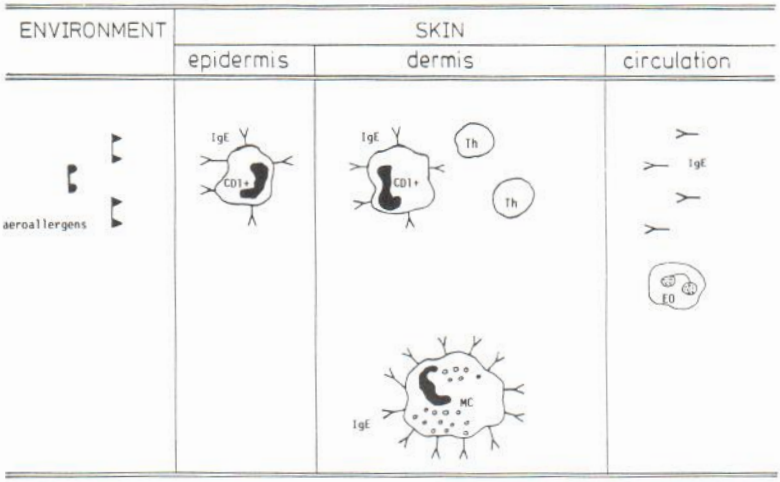

Fig. 1. Inflammatory cells in AD skin. EO = eosinophil; MC = Mast cell; Th = CD4+ cell (T helper/inducer cell); CD1+ = Langerhans cell/indeterminate cell.

Clinically and histopathologically the patch test with aeroallergens induces an eczematous response with spongiosis in the epidermis and a cellular infiltrate in the dermis, mainly consisting of activated T-lymphocytes, IgE+/CD1+ cells and eosinophils (20, 24, 25, 26, 27). The observations made on eosinophils in the patch test reaction to aeroallergens will be discussed later. An influx of basophils was also reported (24), but could not be confirmed in later studies (20, 25, 26, 27). Neutrophils were not present (27).

In conclusion, the patch test with aeroallergens induces an eczematous response, which is specific for AD patients and which has clinical and histopathological similarities with clinically involved AD skin. Therefore, the patch test reaction with aeroallergens forms an attractive working model to investigate the reaction mechanism by which aeroallergens play a role in the pathogenesis of AD. The most important cell types, which are involved in the delayed in time patch test reaction to aeroallergens are IgE+/CD1+ cells, T-lymphocytes and eosinophils.

In vitro lymphocyte response to aeroallergens

Atopic patients have circulating aeroallergen specific T-lymphocytes (28–36). Therefore, we performed lymphocyte stimulation tests with house dust allergen, using epidermal LC as antigen presenting cells to investigate if LC from AD patients can present aeroallergens to T lymphocytes. LC enriched epidermal cell suspensions were prepared from clinically non-involved skin from AD patients and autologous T lymphocytes were obtained from peripheral blood. Commercially available crude house dust extract was used as allergen. T-lymphocytes from AD patients and normal non-atopic controls proliferate to house dust allergen if monocytes from peripheral blood are used as antigen presenting cells. This is in agreement with other reports (28, 35). However, if LC are used as antigen presenting cells, only T-lymphocytes from AD patients show a proliferative response (Table I). Furthermore, the T-lymphocyte proliferative response to house dust allergens, using epidermal LC as

Table I. *Net cpm of T cell proliferation on house dust antigen (50 µg/ml)*

cpm = counts per minute, AD = atopic dermatitis, LC = Langerhans cells, MNC = non T cells from peripheral blood, sIgE on LC = the presence of cell-bound IgE on LC

AD patients	10^3Lc 10^5T cells	sIgE on LC	MNC $(2 \cdot 10^5)$
1	1 452	+	9 593
2	8 034	+	5 106
3	6 205	+	16 142
4	2 140	+	5 242
5	643	+	10 458
6	2 448	+	6 926
7	18 630	+	14 607
8	27 312	+	15 318
9	26 403	+	17 534
10	28	–	10 869
11	–207	–	12 561
12	68	–	472
Non-atopic controls			
1	–140	–	6 386
2	206	–	4 365
3	208	–	6 146

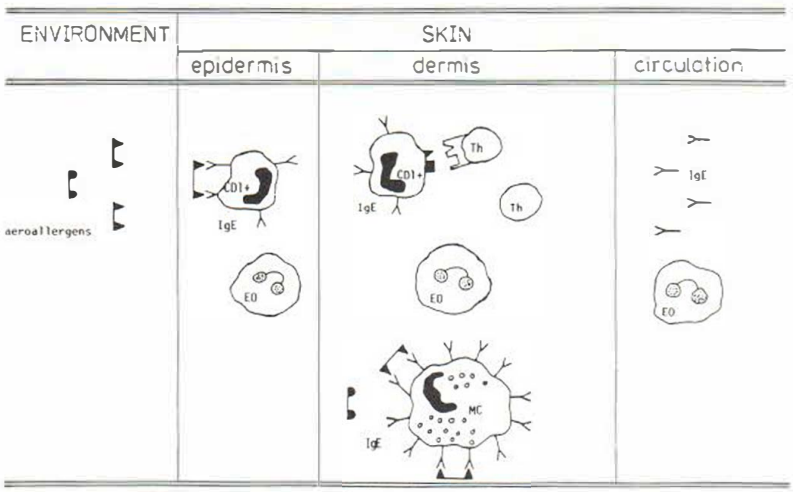

Fig. 2. Inflammatory cells in AD skin during a patch test reaction with aeroallergens.

antigen presenting cells, is restricted to AD patients with IgE-bearing LC. The T-lymphocyte response can be inhibited by anti-HLA-DR antibodies. T-lymphocytes do not react with house dust in the absence of antigen presenting cells. Keratinocytes are not able to induce a T-lymphocyte response to house dust allergen.

These preliminary results suggest that, in contrast to IgE- LC, IgE+ LC from AD patients may be immunologically active and induce a T-lymphocytic response to allergens. However, direct evidence that allergens indeed bind to IgE molecules on LC before presentation to T lymphocytes, is still missing. Furthermore, it is unknown which fraction of the allergen extract is involved in IgE binding and which fraction is recognized by T-lymphocytes. Therefore, studies with more purified allergen fractions are needed.

The possible role of IgE-bearing Langerhans cells

Sofar, arguments have been put forward which suggest that IgE+ LC may play a role in the reaction mechanism behind the patch test reaction to aeroallergens. Aeroallergens may bind to allergen-specific IgE on LC, which present the allergen to T-lymphocytes, inducing an eczematous response (Fig. 2). This mechanism may also be involved in the pathogenesis of the eczematous skin in AD. However, allergen-specific T-lymphocytes, present in AD skin may not only be involved in the eczematous response, but also in the regulation of the IgE production by B-lymphocytes. Two arguments favour this possibility. The first one comes from a recent study of Carswell et al. (37). They reported that in children with AD, with or without asthma, the level of serum IgE antibodies with a specificity to mite body allergen was significantly more elevated than in children with only asthma, whereas the level of serum IgE antibodies with a specificity to the faecal mite allergens was not significantly different. Since the mite body is 12 times greater in size than the faecal particles, these allergens are not likely to be inhaled and it was hypothesized that sensitization to allergens from the mite body occur via penetration of the (eczematous) skin.

A second argument comes from recent work of Hauser et al. (38). They reported that in mice epidermal LC were capable of inducing antigen-specific T-lymphocytes of the TH-2 subtype, which were able to produce Interleukin-4 (IL-4). IL-4 induces the IgE synthesis by B lymphocytes (39). Furthermore, IL-4 induces the expression of a low affinity FC-receptor for IgE not only on B cells (40), but also on monocytes (40). Furthermore, IL-4 induces the differentiation of monocytes into dendritic cells, increases class II MHC expression of monocytes and inhibits the secretion of IL-1 by monocytes (41). If we translate this to AD skin, the following pathway is possible after epidermal contact of aeroallergens with AD skin (Fig. 3).

Aeroallergens are capable of penetrating the skin, bind to IgE on LC and induce a T-lymphocytic response. These T-lymphocytes may be involved in the induction of the eczematous response. However, some T-lymphocytes may belong to the TH-2 subtype, which produces IL-4. IL-4 induces IgE-production by B-lymphocytes in afferent lymph nodes. Furthermore, IL-4 may be involved in the induction of a FcεR on monocytes, which further differentiate into

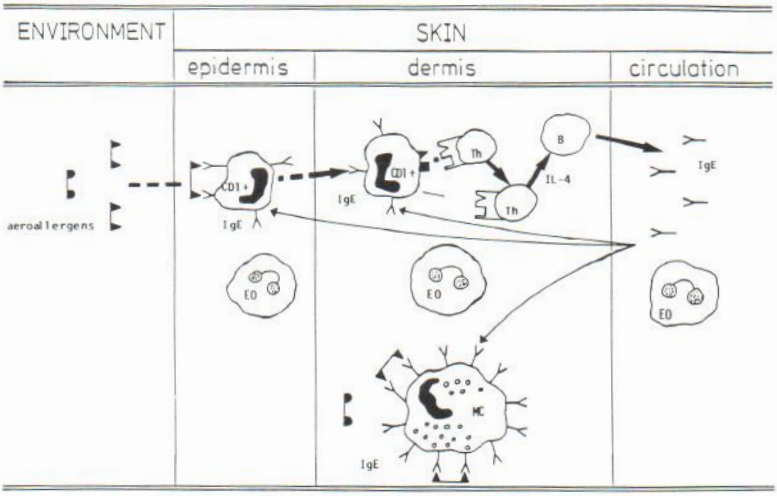

Fig. 3. Induction of IL-4 production after epicutaneous contact with aeroallergens.

dendritic cells, or directly on dendritic cells and LC in the skin, thereby amplifying the response to allergens. In vitro, IL-4 producing T-helper cells are increasingly present after repeated exposure to antigen, suggesting that IL-4 producing cells may play a particular important role in the immune response to antigen that persists or that is encountered repeatedly (42). Contact with environmental allergens like aeroallergens is indeed frequent or even continuous.

IL-4 induces the expression of a low affinity IgE receptor. The IgE receptor on LC seems to have a comparatively high affinity for IgE, since IgE is easily bound in vivo. Therefore, some factors must be involved to increase its affinity for IgE. Good candidates are local inflammatory mediators like platelet-activating-factor, leukotriene B4 and histamine, which have been reported to increase the affinity of the FcεR on eosinophils (43).

The possible role of eosinophils

6–24 h after patch testing with aeroallergens eosinophils are infiltrating the dermis and at 24 h they also appear in the epidermis. At 24 h they are occasionally lying close to IgE+ LC. The eosinophils, lying in the dermis at 6–24 h after patch testing, are in an activated stage since they stain with EG2 antibodies (recognizing eosinophil cationic protein (ECP) from activated eosinophils and also bind to the secreted forms of ECP and eosinophil protein-X (EPX)). However, the eosinophils lying in the epidermis 24 h after patch testing are EG2 negative (27).

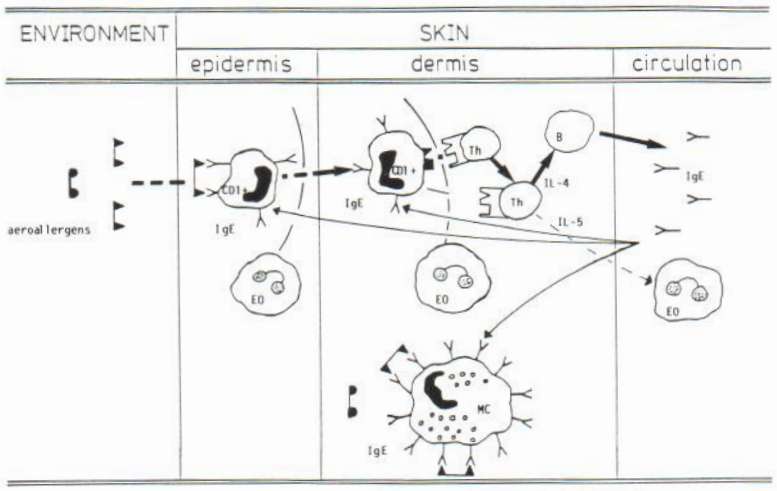

Fig. 4. The role of eosinophils during a patch test reaction to aeroallergens.

If we compare a patch test reaction to aeroallergens with a patch test reaction to conventional contact allergens (thiuram) in the same AD patient, the presence of eosinophils in the epidermis was observed in the 24 h patch test reaction to aeroallergens but not in the thiuram patch test reaction. Therefore, the presence of eosinophils in the epidermis seems to be specific for the patch test reaction to aeroallergens.

The presence of eosinophils may relate the patch test reaction to aeroallergens to the late phase allergic reaction, which may occur after intracutaneous administration of the aeroallergen and is IgE and mast cell dependent. However, a late phase allergic reaction in the skin does not show eczematous changes and is characterized by an infiltration of neutrophils (44, 45). Neutrophils are not observed in the patch test reaction to aeroallergens. This suggests that in the patch test reaction to aeroallergens a reaction mechanism is involved which differs from a classical contact allergic reaction and also from a late phase allergic reaction in the skin.

The TH-2 lymphocyte subtype, which can produce IL-4 is also capable of producing IL-5 (46). Since IL-5 is known as eosinophil colony stimulating factor, this may explain why many AD patients have peripheral blood eosinophilia. In patch test reactions eosinophils are lying in the dermis in mononuclear cell infiltrates and in the epidermis close to LC. Recently, it was reported (47) that eosinophil derived eosinophil cationic protein is capable of inhibiting a T-lymphocyte proliferative response. Therefore, we speculate that eosinophils in patch test reactions to allergens form a defending mechanism of the body to block or inhibit the LC-T cell-B cell amplification pathway (Fig. 4).

In conclusion, these results favour a role for aeroallergens, LC, T-lymphocytes and eosinophils in the pathogenesis of AD. These allergens may after contact with the skin via binding to IgE positive LC induce a cascade of events which may be responsible for the induction of an eczematous response but also for the induction or regulation of the IgE production by B-lymphocytes.

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Langerhans Cell Antigen Presentation and Interleukin-1 Production in Atopic Dermatitis

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We studied Langerhans cell (LC) antigen-presenting capacity and epidermal cell and monocyte interleukin-1 (IL-1) production in 23 atopic dermatitis (AD) patients and 24 healthy controls. Six of the atopics had previously had severe disseminated cutaneous Herpes simplex virus (HSV) type 1 infection but the HSV-induced T cell proliferation was intact in these patients. Five of the AD patients were allergic to birch pollen and had experienced exacerbations of their eczema during the pollen season. The birch pollen induced specific T cell proliferation in two of these 5 AD patients and one of these two also showed a positive patch test reaction to birch pollen. Epidermal cells and monocytes of AD patients produced significantly less IL-1 than those of healthy controls. Further studies are needed to examine whether the impaired IL-1 production in AD is due to a primary defect or results from mediators such as prostaglandins and histamine. ***Key words: Interleukins; Birch pollen; Herpes simplex virus; Atopic eczema.***

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Hereditary and environmental factors and immunological abnormalities play a role in the etiopathogenesis of atopic dermatitis (AD). Recently both type I and type IV hypersensitivity reactions have been connected with AD, and epidermal Langerhans cells (LC) may participate in the development of clinical symptoms of AD (1–5). LC present antigens to T cells (6) and both LC and keratinocytes are capable of releasing immunomodulatory cytokines such as interleukin (IL-1), which contributes to the activation of a variety of cells (7). We therefore examined whether LC functions and the ability of epidermal cells and monocytes to produce IL-1 are abnormal in patients with AD.

MATERIALS AND METHODS

Subjects

The study populations consisted of 23 AD patients and 24 healthy subjects. Within the last year, six of the patients had had at least one severe disseminated cutaneous Herpes simplex virus type I (HSV-1) infection. Five AD patients were included in the study because they had experienced exacerbations during the birch pollen season. All these subjects had positive prick tests and RAST to birch pollen and the proliferation tests were performed in the pollen-free season.

Isolation of cells

Mononuclear cells were obtained by Ficoll-Isopaque centrifugation of venous blood. These cells were used for isolation of T lymphocytes and monocytes. Monocytes were purified on the basis of their adherence to plastic surfaces and T lymphocytes by rosette formation with aminoethylisothiouronium bromide-treated sheep erythrocytes as described before (8). The average purity of monocytes was 94% as assessed by nonspecific esterase staining. T cell populations contained $<0.2\%$ monocytes, $<5\%$ OKT7-positive cells (B lymphocytes) and $>90\%$ OKT3-positive cells (T lymphocytes).

Suction blisters were raised on the uninvolved abdominal skin of patients and healthy subjects. Epidermal sheets were treated with 0.25% trypsin and 0.01% DNase I to obtain crude epidermal cells. Langerhans cells were isolated by attaching them to IgG-coated erythrocyte monolayers in tissue culture dishes (9). Crude epidermal cells contained on the average 2.4% LC and the purity of LC-enriched cells was about 80% as assessed on the basis of OKT6-positive cells.

Langerhans cell – T lymphocyte cultures

T lymphocytes supplemented with 5% LC were stimulated in cultures with HSV-1 (Behringwerke AG, Marburg, FRG) and birch pollen (Aquagen-SQ, Allergologisk Laboratorium A/S, Copenhagen, Denmark) antigens. The cells were suspended in 20% autologous plasma-RPMI 1640 at a density of 0.25×10^6 cells/ml and 0.1-ml volumes of this suspension were pipetted per well of V-bottomed microplates. Thereafter RPMI 1640 (controls) or various concentrations of HSV-1 and birch pollen antigens in RPMI 1640 were added to the plates. The cultures were terminated after 6 days of incubation. Sixteen hours before harvesting 0.125% μCi of iododeoxyuridine was added per well. The uptake of the isotope was measured with a gamma counter. The results were expressed as stimulation indices (SI). SI = uptake of isotope in stimulated culture/uptake of isotope in nonstimulated control culture.

Epidermal cell and monocyte cultures

For the production of IL-1 crude epidermal cells or purified monocytes (10^6 cells/ml) were incubated in RPMI 1640 supplemented with 5% autologous plasma or AB serum for two days. To enhance the elaboration of IL-1, lipopolysaccharide (LPS, final concentration 5 μg/ml), 4β-phorbol 12-myristate 13-acetate (PMA, final concentration 20 ng/ml) or formalin

Table I. *Generation of interleukin 1 by epidermal cells and monocytes from atopic patients and health controls*

Cells	Atopic patients		Healthy controls		Significance (Mann-Whitney U-test)
	n	IL-1 (U/ml)	*n*	IL-1 (U/ml)	
Epidermal cells	15	6.7 (1–14)[a]	9	13.3 (5–26)	$p<0.01$
Monocytes	19	19.8 (10–49)	21	38.4 (20–88)	$p<0.01$

[a] Mean (range).

treated *Staphylococcus epidermidis* bacteria (bacterium-to-cell ratio 2:1) were added to the cultures. IL-1 activity in the culture supernatants was determined as described by Luger et al. (10) using the thymocyte comitogenicity assay. Various concentrations of a standard IL-1 preparation (Genzyme, Norwalk, CT, USA) were also included and the results expressed as U/ml.

Patch tests

These were performed on the upper arm by using 0.1 ml of birch pollen allergen (10^5 SQ units/ml, Aquagen, Allergologisk Laboratorium A/S) in Finnchambers®, as described by Reitamo et al. (2). A diluent was used as a negative control. The tests were removed at day 2 and also read on day 3.

RESULTS

Both epidermal cells and monocytes from atopic patients produced clearly less IL-1 than those from healthy subjects ($p<0.01$, Table I). In addition, monocytes elaborated about three times higher IL-1 activities than epidermal cells both in atopics and healthy controls (Table I).

Table II shows the results on antigen presentation by epidermal LC from atopic and healthy subjects. The capacity of LC to present HSV-1 to T lymphocytes was intact in atopic patients with previous HSV infections. All these persons were seropositive to HSV-1 whereas there were a few seronegative individuals among other atopic patients and healthy subjects. The cells from the seronegative persons were not stimulatd with HSV in cultures.

Birch pollen appeared to be a weak lymphocyte stimulant in cultures. It induced T cell proliferation (SI $\geqslant 2.5$) in 2/5 patients with allergy to this antigen. The patch test to birch pollen was positive in 1/5 AD patients and showed an eczematous reaction with a maximum at day 3. This patch test-positive patient exhibited the highest in vitro response to birch pollen.

DISCUSSION

In the present study both monocytes and epidermal cells from AD patients produced lower IL-1 activities than the cells from healthy controls. These results are consistent with those of Mizoguchi et al. (11) who measured venous blood monocyte-derived IL-1 in atopics and normal controls. On the other hand, en-

Table II. *Presentation of Herpes simplex virus type 1 and birch pollen antigens to T lymphocytes by epidermal Langerhans cells*

Study group	Antigen[a]			
	HSV		Birch pollen	
	n	SI[b]	*n*	SI
Atopic patients				
With HSV infections	6	90.1 (48.2–180.9)		n.d.
With birch pollen allergy	5	32.6 (0.8–141.6)	5	2.1 (0.5–3.6)
Healthy controls	7	36.2 (2.3–130.4)	2	1.1 (0.9–1.3)

[a] Results with optimal antigen concentration (10^{-2}–10^{-3} dilutions of a stock preparation of HSV-1, 10–100 μg/ml of birch pollen allergen).
[b] Mean stimulation index (range).

hanced releasability of inflammatory mediators such as histamine, prostaglandin E_2 and leukotrienes B_4 and C_4 has been shown to occur in AD (12, 13). In vitro experiments indicate that both prostaglandin E_2 and histamine are able to suppress the elaboration of IL-1 (14–16), whereas leukotrienes B_4 and D_4 enhance IL-1 production (17). One explanation for the observed impairment in epidermal cell and monocyte production of IL-1 in AD might be the increased amounts of inflammatory mediators with IL-1 suppressive activity, but further studies are needed to confirm this.

We did not find any defect in the ability of epidermal LC to present HSV to T lymphocytes when our AD patients were investigated 1–12 months after the last episode of a severe HSV infection. However, some alterations seem to occur during the acute infection. El Araby et al. (18) and Vesley et al. (19) have measured HSV-induced proliferation of peripheral blood mononuclear cells in subjects with severe recrudescent HSV infections. These investigators reported diminished proliferative responses during the acute HSV attack and several months afterwards in patients with widely disseminated eczema herpeticum.

Several pieces of evidence suggest that environmental antigens may play a role in the pathogenesis of AD. Food, pollen, animal dander and house dust mite antigens have been shown to induce positive patch tests reactions and are suspected to exacerbate AD (2, 4, 5). We examined five such patients and found that the birch pollen antigen induced proliferation in 2/5 cultures and the patch test was positive in 1/5 cases. Our results suggest that the epidermal LC of at least strongly birch pollen allergic AD patients are capable of presenting this allergen to T cells, and such a mechanism could explain the AD exacerbations during the pollen season.

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IgG Anti IgE in Atopic Dermatitis

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Autoantibodies specific for immunoglobulins have been demonstrated in sera of patients with various diseases, but also in healthy subjects, in particular after antigenic stimulation, with increasing incidence with age. In some diseases, particularly rheumatoid arthritis, these antiglobulins have been well characterized, and their participation in immune complex formation with serum immunoglobulins of the corresponding specificity has been confirmed. In 1971 Williams demonstrated that sera from allergic patients contained circulating IgM antibodies directed against IgE (1). Subsequently, different authors reported the occurrence of IgG autoantibody to IgE in sera from patients with asthma, atopic dermatitis, rhinitis and chronic urticaria (table 1, also for references 1–8). Atopic dermatitis (AD) is a common skin disorder characterized by several immunological abnormalities. Although the pathogenetic role of IgE in the inflammatory response of AD remains uncertain, there is universal agreement that IgE bound to basophils and mast cells, once bridged by a specific antigen, trigger the release of histamine and other mediators to initiate the inflammatory response (9). We now report evidence for the presence of anti-IgE autoantibody both free and complexed with IgE in sera from patients with AD, its possible role in the pathogenesis of allergic diseases and its interference in total and specific IgE determinations.

METHODS

To measure the IgG anti-IgE antibody we used an ELISA assay. Briefly, a 96 wells microtiter plate was coated with purified myeloma IgE (ADZ). After washes, sera were added at different dilutions. After 18 h at room temperature and three more washes, alkaline phosphatase conjugated immunosorbent purified goat anti-human IgG (Zymed) was added. After washes, the substrate was added and after 30 min of color development, the optical density (OD) was read at 405 nm using a Titertek Multiskan spectrometer. Our results were also referred to a standard curve obtained with purified IgG anti IgE to estimate the amount of IgG anti IgE of the sample. We obtained the purified IgG anti IgE from the serum of a child with atopic eczema. 3 ml of serum were passed on an immunosorbent Sepharose column (dimensions 1.2×5 cm) coated with purified IgE. Immunosorbent bound IgG anti IgE were eluted with glycine HCl buffer 0.2 M, pH 2.8 and the pH quickly readjusted by addition of 2 M NaOH. The total content of IgG of the eluted antibody was measured, and the concentration adjusted to 1 mg/ml. IgE content was checked by paper radio immunosorbent assay (PRIST) and was below 100 pg/ml. This purified IgG anti IgE was also used to assess its interference with PRIST and radioallergosorbent (RAST) tests. The effect on measurements was tested by adding tenfold dilutions of the purified preparation of this autoantibody either to sera and standard (keeping constant the final volume incubated with paper discs) or to the radiolabelled anti-IgE reagent.

RESULTS

The level of IgG anti IgE but not of IgM anti-IgE were elevated in 14 out of 18 sera (Fig. 1). Significant IgG anti-IgE activity remained in 12 sera after adsorption over pooled human IgG F(ab')2 Sepharose. The IgG anti-IgE activity appeared to be directed toward the Fc portion of IgE because the absorption of positive sera over IgE (PS)-Sepharose but not over myeloma IgG Sepharose completely removed their reactivity with IgE (ADZ) and because the autoantibody reacted against the protein backbone of the FC portion of IgE synthesized from a fragment of the cloned gene of human myeloma IgE (ND) heavy chain (6). Fractionation of sera by gel filtration revealed that the IgG anti IgE activity was present both as monomeric IgG and in IgE containing immune complexes (Fig. 2). We also probed the ability of IgG anti IgE to inhibit IgE recognition by heteroantisera to IgE used in commercially available PRIST and RAST assays. Our results indicate that IgG anti IgE decreases total IgE results by 10–90% according to its weight ratio to IgE molecules present in samples devoid of endogeneous anti-IgE. Sera with high content of specific IgE to house dust mite are little affected by anti-IgE, but RAST class 2 sera can turn to negative when purified IgG anti IgE is added.

DISCUSSION

We have previously published evidence of elevated levels of IgG anti-igE antibodies in sera of allergic patients (6). In the present study we extend our obser-

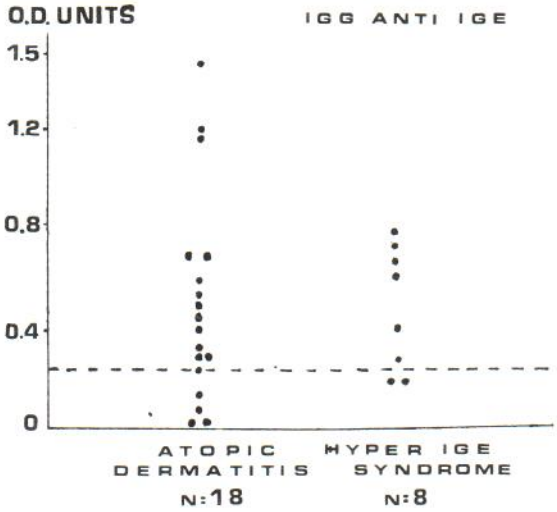

Fig. 1. IgG anti IgE (O.D.) in patients with atopic dermatitis and Hyper IgE syndrome.

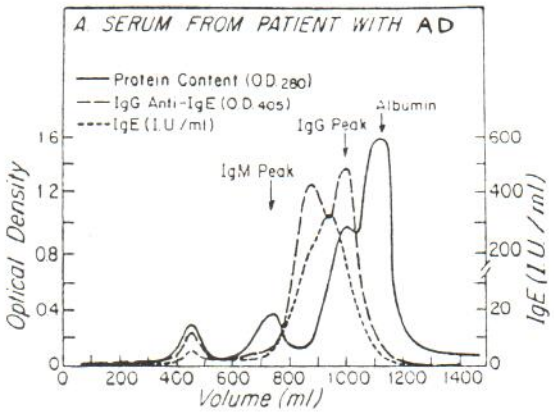

Fig. 2. Representative gel fractionation of a serum sample from a patient with atopic dermatitis. The sample was passed through a Sepharose 6B column and IgE (U.I./ml) and IgG anti IgE (O.D.) were determined in the column fractions.

vation to patients with atopic dermatitis. The epsilon chain specificity of IgG anti IgE was confirmed by competitive inhibition by its reactivity with the Fc portion synthesized from a fragment of the cloned gene of human myeloma IgE heavy chain. High titers of antibodies to isotypic determinants of IgE have been generated by immunization of mice (10) and rats (11) with syngeneic IgE. In contrast to conventional rheumatoid factors, the anti-IgE antibodies are of moderately high affinity. In rats the induction of auto anti-IgE inhibited total and specific IgE levels and had a degranulating effect on mast cells. According to our gel filtration results, we found that the anti-IgE autoantibody was present in monomeric form, but also as immune complexes with IgE. Only the monomeric form of IgG anti IgE from a patient with AD showed the capacity to trigger mast cells and basophils to release histamine in vitro (data not shown). When the same autoantibody was intradermally injected in a healthy adult an immediate wheal and flare was obtained. In five patients we could not separate the IgG anti IgE from the complexes with IgE: the complexed form was incapable of releasing histamine from basophils and PGD2 from lung mast cells (12). The dysfunction of cell mediated immunity in AD correlates with the severity of skin lesions and the levels of serum IgE. Large size immune complexes may contribute to the impairments of cell mediated immunity, chemotaxis defect and to inflammatory skin lesions associated with atopic dermatitis. Our data, moreover, demonstrate that IgG anti-IgE antibody can affect both total and specific IgE determinations: we suggest that its presence should be considered in evaluating the laboratory results.

Table I. *Summary of published studies on anti-IgE antibodies*

			(Ref.)
1972	Williams	IgM anti IgE in allergic disorders	1
1981	Inganas	IgG anti IgE in allergic asthma	2
1984	Nawata	IgG anti IgE in bronchial asthma	3
1985	Johansson	IgG anti IgE in atopic subjects	4
1985	Nawata	IgG anti IgE in atopic dermatitis	6
1986	Quinti	IgG anti IgE in atopi syndromes	7
1986	Paganelli	IgG anti IgE in Hyper IgE syndrome	8
1988	Gruber	IgG anti IgE in urticarial syndromes	9

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Histamine and Atopic Eczema

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Apart from increased production of immunoglobulin E antibodies and disturbed T-cell regulation, altered patterns of releasability of vasoactive mediators have been described in patients with atopic exzema. The best studied substance is histamine which is a classical inducer of pruritus in man. Elevated concentrations of histamine have been found in vivo in the skin and in the plasma of patients with atopic exzema especially during exacerbation of the disease. Similar findings have been described for other atopic diseases as extrinsic bronchial asthma. Histamine acts via characteristic receptors; symptoms as itch, wheal formation, mucus production, contraction of smooth muscle, tachycardia and hypotension are mediated via H1-receptors, while H2-effects include acid secretion in the stomach as well as the development of flush and itch reactions, blood pressure changes and cardiac arrhythmia. Of special interest is an inhibitory effect of histamine on lymphocyte reactions mediated via a H2-receptor. The existence of a new H3-receptor in the brain serving as autocrine feed-back inhibitor of histaminergic neurones has been established in the rat but not yet in man. In vitro an increased histamine releasability of peripheral leukocytes has been found after stimulation with a variety of different substances. The difference between patients with atopic eczema and normals is generally most pronounced after stimulation with anti-IgE. There is, however, a tendency towards an increased spontaneous histamine release compared to normals. The release reaction of histamine seems to occur more rapidly in atopics compared to normals. Among possible factors influencing histamine releasability the imbalance in the cyclic nucleotide system (increased response of cGMP to cholinergic stimulation and decreased response of cAMP to β-adrenergic stimulation) might play a pathogenetic role. Arachidonic acid metabolites known to regulate histamine release (PGE_2 inhibits histamine release while cyclooxygenase blockers enhance histamine release and lipoxygenase blockers inhibits histamine release) also may be of relevance. Histamine definitely is not the only relevant mediator substance in the pathophysiology of atopic eczema; it may, however, serve as a marker of mast cell and basophil activation. Clinical trials with various antihistamines have shown some therapeutic benefit in the management of atopic eczema patients. Future studies in the field of mediator research may lead to new therapeutic approaches for the treatment of atopic eczema.

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HISTAMINE EFFECTS

Although known for almost 80 years, histamine still remains a fascinating substance for many researchers (7, 8, 17, 18, 28, 46, 47, 53, 69, 83). The definition of the physiological role of histamine in health and disease remains incomplete. We know that histamine exerts powerful effects mainly via two receptors (Table I). The description of a new H3-receptor in the brain deserves special interest (4, 81 *a*).

The role of histamine as a mediator of immediate-type hypersensitivity diseases (both allergic and pseudo-allergic in origin) is quite well established (7, 16, 17, 36, 68, 79). Similarly well defined is the H2-mediated role of histamine in the induction of gastric acid secretion.

Recent interest has focussed on anti-inflammatory effects of histamine as a modulator of immune reactions acting predominantly on H2-receptors on the surface of leukocytes there by inhibiting a variety of immune reactions (Table II) (9, 13, 72, 73, 81, 85).

In a study in 16 patients with atopic eczema we found an inhibitory effect of histamine upon pokeweed-mitogen(PWM)-induced lymphocyte stimulation (Fig. 1). This effect was shown to depend upon the presence of T8-lymphocytes in the cell suspension, a shown in Fig. 2: after depletion of T8-lymphocytes the inhibitory effect of histamine upon PWM-induced lymphocyte stimulation was no longer demonstrable in atopics nor in controls.

Role of histamine in the pathophysiology of atopic eczema

In spite of great progress in experimental and clinical allergology and dermatology in the last decades the

Table I. *Histamine effects in human organs*

Organ	Stimulatory	Inhibitory	None/ negligible
Vessels			
Large veins (>80 μm)	+	–	–
Arterioles, venules (20–30 μm)	–	+	–
Permeability of "capillaries" (postcapillary venules)	+	–	–
Extravascular smooth muscle			
Bronchi, gut	+	–	–
Uterus, bladder, gallbladder, iris	–	–	+
Stomach (secretion)	+	–	–
Salivary glands	+	–	–
Heart			
Rate, force, output	+	–	–
AV conduction	–	+	–
Ventricular arrhythmia	+	–	–
Nervous system			
Sensory fibers	+	–	–
Central effects	(+)?	–	–
Endocrine system			
Adrenal medulla	–	–	+
Leukocytes	–	+	–

pathogenesis of atopic eczema is still not well established.

Research interest has focussed on mainly three aspects:

increased production of immunoglobulin E,

disturbed T-cell regulation,

altered pharmacological reactivity and releasability of vasoactive mediators (6, 11, 15, 23, 25, 26, 31, 32, 35, 41, 42, 43, 44, 48, 56, 57, 58, 62, 63, 80, 85, 94).

Previously we have advanced the concept of a "vicious cycle" of this different factors acting together in the pathophysiology of atopic eczema (61).

The best studied mediator substance is histamine which is a classical inducer of pruritus in man (57, 69). Elevated concentrations of histamine have been found in vivo in the skin and in the plasma of patients with atopic eczema especially during exacerbation of the disease (37, 38, 66, 74) (Table III). Similar findings have been described for other atopic diseases as extrinsic bronchial asthma (82). In vitro an increased histamine releasability of peripheral leukocytes has been found after stimulation with a variety of different substances by many other authors (5, 14, 15, 22, 51, 65, 69, 74, 90). The difference between patients with atopic eczema and normals is generally most pronounced after stimulation with anti-IgE. There is, however, a tendency towards an increased spontaneous histamine release compared to normals (69, 84). The release reaction of histamine seems to occur more rapidly in atopics compared to normals (91).

Among possible factors influencing histamine releasability the imbalance in the cyclic nucleotide system (increased response of cGMP to cholinergic stim-

Table II. *Immune reactions inhibited by histamine*

Mast cells and basophils (mediator release)
Neutrophil function (chemotaxis, phagocytosis, enzyme release)
Eosinophils
Macrophages (complement production)
Lymphocyte proliferation and lymphokine production
Lymphocyte cytotoxicity
Concanavalin A-induced suppressor cell activity

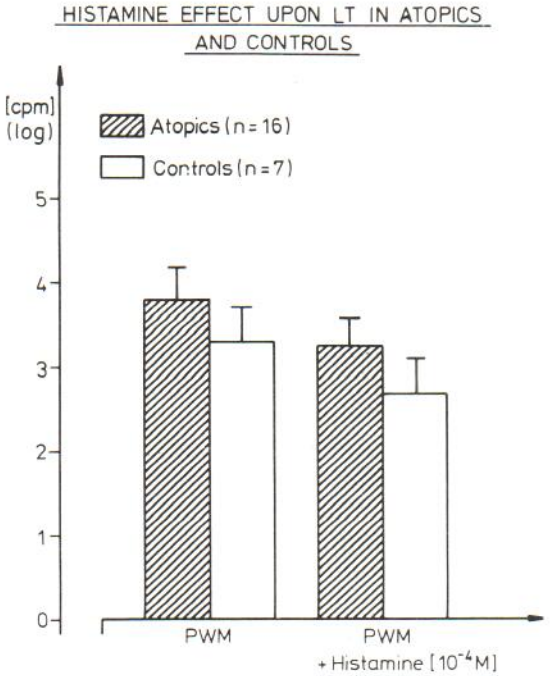

Fig. 1. Effect of histamine added to lymphocyte cultures upon lymphocyte transformation (LT) induced by pokeweed mitogen (PWM) in patients with atopic eczema and controls.

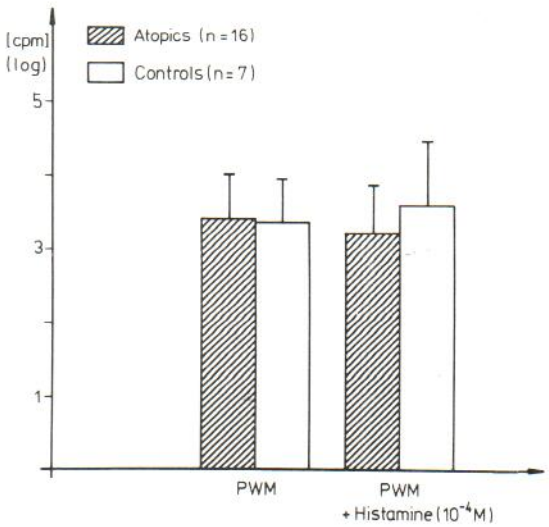

Fig. 2. Effect of histamine added to lymphocyte cultures upon lymphocyte transformation (LT) induced by pokeweed mitogen (PWM) in patients with atopic eczema and controls after specific depletion of T8-cells by rosetting technique with monoclonal antibodies (see ...)

ulation and decreased response of cAMP to β-adrenergic stimulation) (1, 3, 12, 14, 21, 27, 34, 40, 43, 49, 54, 59, 64, 65, 78, 86, 87, 88) might play a pathogenetic role.

Arachidonic acid metabolites known to regulate histamine release (PGE_2 inhibits histamine release while cyclooxygenase blockers enhance histamine release and lipoxygenase blockers inhibits histamine release) (2, 16, 18, 19, 20, 50, 69, 91) also may be of relevance.

Histamine definitely is not the only relevant mediator substance in the pathophysiology of atopic eczema; it may, however, serve as a marker of mast cell and basophil activation. Similar results of increased formation of leukotriene B_4 in involved skin of patients with atopic eczema (75) as well as enhanced in vitro leukotriene B_4 secretion from peripheral leukocytes in atopic patients have been reported (76).

Psychosomatic interactions, histamine and atopic eczema

The in vivo and in vitro demonstrable dysregulation of the autonomic nervous system in patients with atopic eczema (33, 43, 44, 56, 68, 94) together with the role of autonomic nervous system transmitters in regulating histamine release (inhibition by β-adrenergic, enhancement by cholinergic stimuli) (27, 46, 64, 65, 78, 86) and the possible existence of a newly described H3-receptor in the brain might open a new field of research in order to more clearly define the nature of psychosomatic interactions in this disease (10, 67, 92). This new H3-receptor has been demonstrated in the rat brain where it serves as an autocrine feed-back inhibitor of histaminergic neurones leading

Table III. *Histamine and atopic eczema*

In vivo
Histamine concentration elevated in the skin (±)
Histamine elevated in plasma (during exacerbation)
In vitro
Altered releasability
Increased release from basophil leukocytes
Increased speed of release
Influence of autonomic nervous system transmitters
Modulation by arachidonic acid metabolites

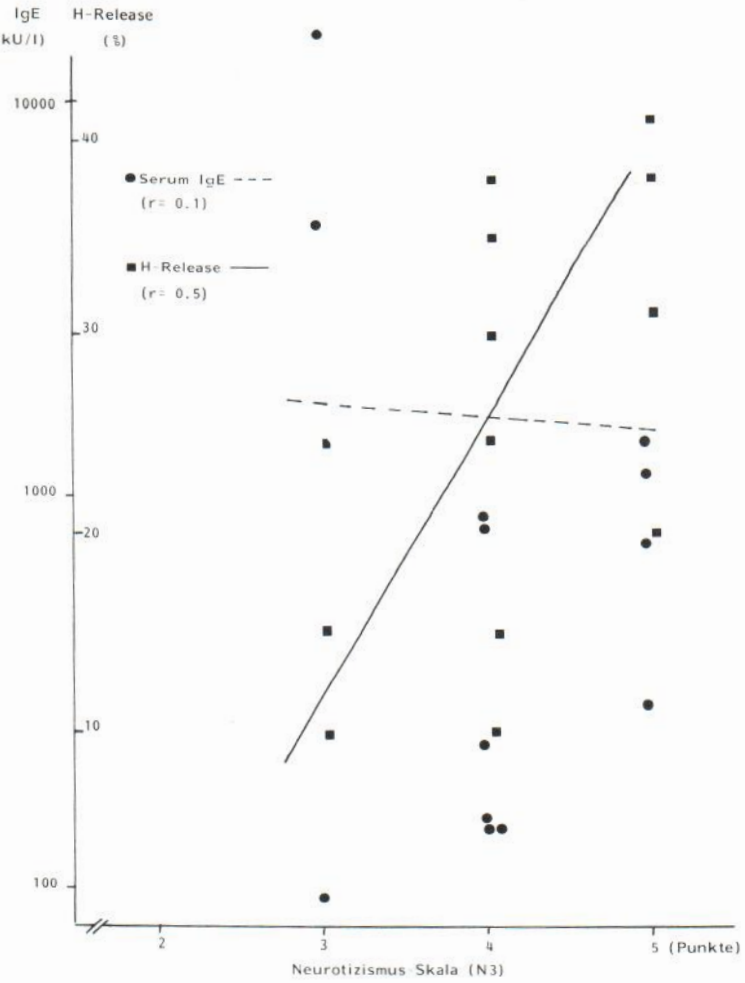

Fig. 3. Correlation of psychodiagnostic test results in the neuroticism scale of the "Hamburg Neuroticism and Extraversion Scale" (HANES) for children and adolescents to somatic findings as serum IgE concentration and in vitro histamine releasability after anti-IgE-stimulation of peripheral leukocytes.

to diminished histamine synthesis in and synaptic secretion from these cells (4, 81 *a*).

It has been shown by various authors that histamine release can be induced by stress in various forms (60, 69).

In a psychosomatic investigation using several psychodiagnostic tests in children with atopic eczema and control children with non-inflammatory dermatologic diseases we compared the results of the "Hamburg Neuroticism and Extraversion Scale" (HANES) for children and adolescents with somatic findings as extent of skin lesions, serum IgE-level and in vitro histamine releasability (67). As shown in Fig. 3 there was no correlation between the results of the psychodiagnostic tests to any somatic finding except for the slightly pronounced positive correlation between in vitro histamine releasability towards anti-IgE and neuroticism as measured in the HANES scale (Fig. 3).

Therapeutic approaches

Therapeutic approaches to histamine-mediated diseases can act at different stages (Table IV) from the inhibition of histamine synthesis via blockade of histamine release at different levels until specific antagonism of histamine effects (Table IV) (11, 24, 58, 68, 94).

In antihistamine therapy new developments include the production of non-sedating H1-antagonists, the combination of H1- and H2-antagonists as well as the application of H1-antagonists with mast cell blocking activity (Table V).

The side effects of classical H1-antagonists include mostly sedative effects; it seems of interest, however, that in double-blind studies these sedating side effects are observed regularly, especially in a certain percentage of atopic patients even under placebo (Table VI).

There is some controversy regarding the effect of

Table IV. *Therapeutic modalities in histamine-mediated diseases*

Histamine synthesis inhibitors
Histamine release blockers
cAMP-active substances
Flavonoids
Ca-antagonists (?)
Inosiplex (?)
Lipoxygenase inhibitors
Histamine antagonists

Table V. *Antihistamine therapy*

H1-antagonists (classic)
H1-antagonists (non-sedating)
H1-antagonists with mast cell blocking activity
H2-antagonists
H1- + H2-antagonists combined
H3-agonists or antagonists (?)

Table VI. *"Antihistamine"-side effects under placebo treatment (%)*

Sedation	13
Headache	6
Dryness of mouth	5
Diarrhoea	2
Nausea	2
Abdominal pain	2
CNS-stimulation	1
Exanthema	1
Increase in body weight	1

Table VII. *Antihistamines, itch and atopic eczema*

H1/H2 antagonists not superior to H1 or H2 alone (Foulds & Mackie, 1981)
Terfenadine, astemizol (1 dose) no effect upon endogenous, but upon peripherally induced itch (Krause & Shuster, 1983)
H1/H2 not superior to H1 but positive trend regarding itch (Frosch et al., 1984)
Histamine antagonism independent of sedation (Hägermark et al. 1985)
Tazyfylline no effect upon itch and scratch response in atopic eczema (Savin et al., 1986)
Tazyfylline dose-dependent effect upon peripherally induced itch (Ring et al., 1988)
Oxatomide effective in atopic eczema (placebo control) (Weinberg & Leaver, 1987)
Terfenadine, Ketotifen both effective equally (Tholen et al., 1987)
Subjective feeling of sedation independent of objective parameters (driving performance) (Ring & Bieber, 1987)
Dimetinden, astemizol equally effective (Kiehn & Rakoski, 1987)

antihistamine therapy in atopic eczema. Especially with regard to the question whether sedating side-effects are essential for a possible therapeutic effect of antihistamines. In Table VII some studies from the literature are enlisted dealing with the efficacy of antihistamines upon itch or atopic eczema.

Obviously more studies will have to be done to really answer these questions. So far it seems to be fair to state that antihistamines can never represent the one and only therapeutic modality in this disease; on the other hand antihistamines have their place among many other therapeutic approaches in the treatment regimen of atopic eczema (11, 56, 68, 94).

New approaches include the application of mast cell blockers like oral cromoglycate, where we found some beneficial effect in an open clinical trial especially in patients with evidence for food allergy (45).

The modulation of fatty acid metabolism, either by giving gamma-linoleic acid (77, 93) or eicosapentainoic acid (EPA) is under investigation. Our results with a double-blind controlled study with EPA in atopic eczema showed no effect compared to placebo (to be published).

Future studies in the field of mediator research may lead to new therapeutic approaches for the treatment of atopic eczema.

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Acta Derm Venereol (Stockh) 1989; Suppl 144: 78–82

Family Background of Respiratory Atopy: a Factor of Serum IgE Elevation in Atopic Dermatitis

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The incidence of family history of atopic diseases in patients with atopic dermatitis (AD) was dependent upon the number of family members. In AD patients whose family had four persons or less, the diagnostic value of atopic family history was considerably diminished. To investigate if personal or family history of atopic respiratory disease (ARD) are implicated in elevating serum IgE in AD, serum IgE values were analyzed in 200 AD patients who had a family of five or more. AD patients who had a personal or family history of ARD showed significantly higher serum IgE than AD patients who lacked both personal and family ARD history. Patients with only AD and very high serum IgE commonly had a family ARD history. Patients with severe AD and normal serum IgE mostly lacked personal and family ARD history. It appears that about 40% of total AD patients do not have predisposition for ARD. ***Key words: Heterogeneity of serum IgE; Family history of atopy; Atopic respiratory disease; Severity of dermatitis.***

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Although the elevation of serum IgE level in many patients with atopic dermatitis (AD) has been well established, the diagnostic value of elevated serum IgE for this condition is still obscure (1–3). The main reason for the obscurity seems to be the difficulty in explaining the fact that serum IgE levels are normal in 20% to 30% of patients with otherwise typical AD (4–6).

Various investigators (4–10) have demonstrated that severity of dermatitis and coexistence of respiratory atopic disease (ARD) are the important clinical factors which elevate serum IgE concentration in AD. However, all previous studies have confirmed that serum IgE levels are not elevated in some patients with severe AD, and that very high serum IgE values are often observed in patients with AD who do not have a personal history of ARD. These findings might suggest that some other factors are also implicated in elevating serum IgE in AD.

By examining influences of family background of ARD on serum IgE concentration in patients with AD, I previously reported that the presence of a family history of ARD plays an important role in serum IgE elevation in AD (11). The present paper summarizes the data on the relationship between family history of ARD and serum IgE levels in a large number of patients with AD.

Family history of atopy in recent years

Several decades ago, it was reported that approximately 70% of patients with AD had a family history of atopic diseases (12, 13). Therefore, atopic family history has been regarded as one of the major criteria in the diagnosis of AD (1, 14).

Unfortunately, the incessant decrease in the birth rate through the last decades has greatly lowered the incidence of family history of atopic diseases. Table 1 shows the relation between the number of family members and family history of atopy in 427 consecutive young adults (age: 15–30 years) with AD who visited our dermatological clinic during the last three years (1985–1987). The most prevalent family was that of four persons, i.e., parents and two children. In patients with AD who had such a small nuclear family, the incidence of atopic family history was only 53%. On the other hand, family history of atopy was

Table I. *Relationship between number of family members and incidence of atopic family history in 427 patients with atopic dermatitis (age: 15–30 years)*

No. of family members	No. of patients	Family history of atopic diseases +	–
3	48	15 (31%)	33 (69%)
4	251	134 (53%)	117 (47%)
5	101	76 (75%)	25 (25%)
6–7	27	21 (79%)	6 (21%)
Total	427	246 (58%)	181 (42%)

positive in more than 70% of patients with AD who had a family of five or more. Thus, it is clear that data on atopic family history has reliable diagnostic value only in those patients with AD who have a family of five persons or more.

Classification of patients with AD

To investigate whether presence of family ARD history implies a relationship to serum IgE level in AD, 200 consecutive young adults (age: 15–30 years) with AD, 100 mild cases and 100 severe cases, were selected. The present study examined only the patients with AD who had a family of five or more.

The degree of dermatitis was determined using the following criteria: *Mild:* localization of active skin lesions to two or three anatomical areas for at least 6 months prior to the present examination. *Severe:* involvement of 70% or more of the total body surface for at least 6 months before this study.

The 200 patients were classified into three groups: 1) those who had personal ARD history (98 cases), 2) those who did not have personal ARD history, but had a family history of ARD (40 cases), and 3) "pure" AD patients who had neither personal nor a family history of ARD (62 cases). The distribution of the three groups in mild AD cases was almost the same to that in severe AD cases (Table II).

Evaluation of serum IgE levels

Serum IgE concentrations were measured by the radio-immunosorbent assay (Pharmacia, Uppsala), and expressed in U/ml. Mikawa et al. (15) reported that the upper limit of serum IgE levels in normal Japanese adults is around 500 U/ml. In the present study serum IgE values greater than 500 U/ml were regarded as increased, and those greater than 2000 U/ml as very high.

Table II. *Classification of patients with atopic dermatitis (AD) by personal/family history of atopic respiratory disease (ARD)*

	Patients with AD who had a personal ARD history	Patients with only AD who had a family ARD history	"Pure" AD patients
Mild AD (100 cases)	49	21	30
Severe AD (100 cases)	50	18	32

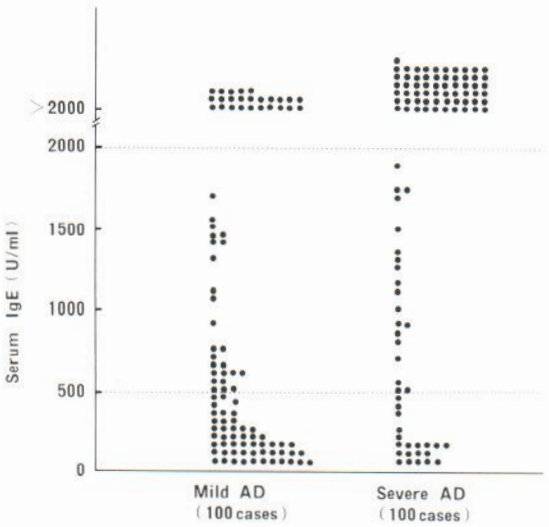

Fig. 1. Distribution of serum IgE values in 100 mild cases and 100 severe cases of atopic dermatitis.

Serum IgE levels in mild AD cases and severe AD cases

Fig. 1 shows the distribution of serum IgE values in the 100 mild cases and 100 severe cases of AD examined. The levels of serum IgE roughly correlated with the severity of dermatitis. However, a very high serum IgE value was observed in 25% (25/100) of the mild cases, while a normal or only moderately elevated serum IgE level (i.e., lower than 2000 U/ml) was seen in 39% (39/100) of the severe cases.

Serum IgE levels in AD patients having a personal ARD history

In the group of patients with AD who had a personal history of AD, serum IgE levels were elevated in the majority of mild cases, and in all of the severe cases (Fig. 2). A feature of this group was that very high serum IgE values were obtained in nearly all (47/49) of the severe cases, and in a considerable number (17/38) of the mild cases. Overall, there was a positive correlation between the levels of serum IgE and the severity of dermatitis.

Serum IgE levels in patients with "pure" AD

In the group of patients with "pure" AD who had neither a personal nor a family history of ARD, serum IgE levels again correlated with the severity of dermatitis (Fig. 3). But the severe cases of this group showed only a slight or moderate elevation of serum IgE. Thus, as can be seen from Figs. 2 and 3, there was a

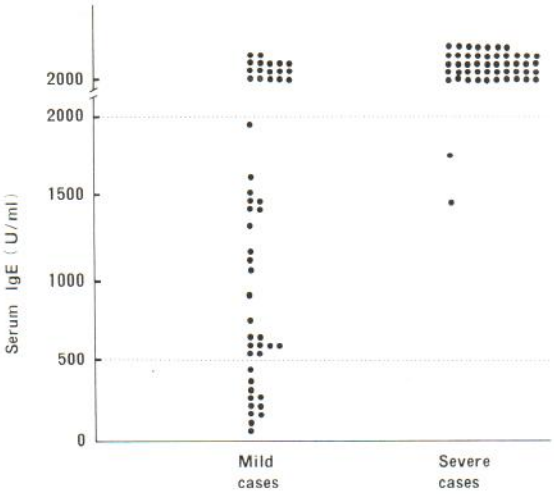

Fig. 2. Serum IgE levels in patients with atopic dermatitis who had a personal history of respiratory atopy.

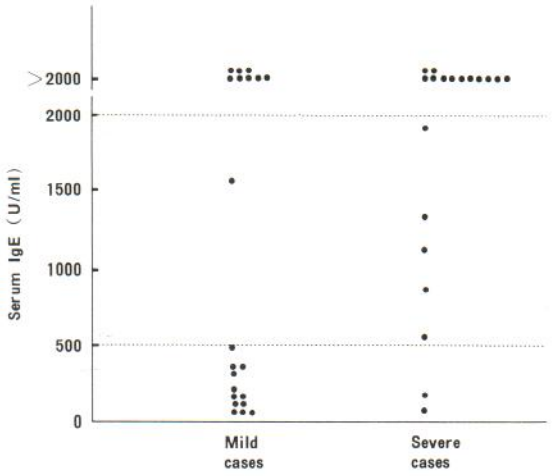

Fig. 4. Serum IgE levels in patients with only atopic dermatitis who had a family history of respiratory atopy.

striking difference in the magnitude of serum IgE elevation between patients with severe AD who had a personal history of ARD and severe cases of "pure" AD.

An important finding was that serum IgE levels were normal in 20 (63%) of the 32 severe cases in the "pure" AD group. It then became evident that most (20/22) of the patients with severe AD and a normal serum IgE value belonged to the "pure" AD group (Figs. 1 and 3).

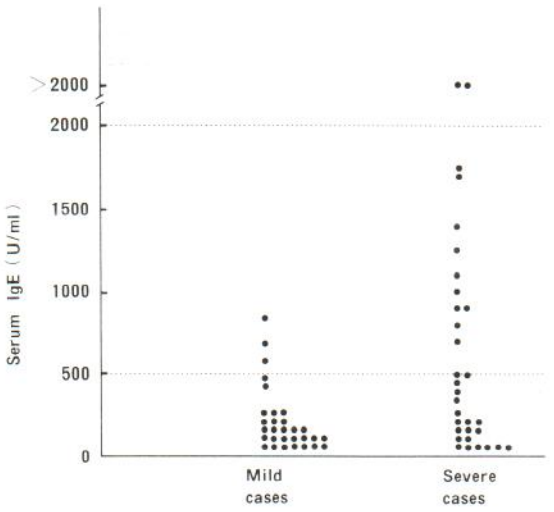

Fig. 3. Levels of serum IgE in patients with "pure" atopic dermatitis who had neither personal nor family history of respiratory atopy.

Serum IgE levels in AD patients having only a family history of ARD

In patients with AD who did not have a personal but had a family ARD history, serum IgE level and severity of dermatitis had a close correlation (Fig. 4). An interesting finding was that in contrast to the "pure"

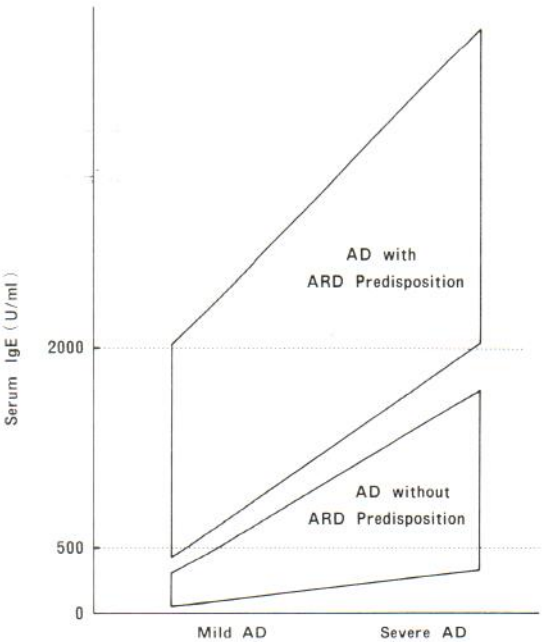

Fig. 5. A schematic presentation of the heterogeneous distribution of serum IgE values in patients with atopic dermatitis.

AD group, half (20/40) of the patients in this group showed very high serum IgE levels. Thus, it became clear that the great majority (20/22) of the patients with only AD and very high serum IgE value were those who had a family history of ARD (Figs. 3 and 4).

DISCUSSION

The present results confirm the findings of previous studies (4–10) that serum IgE levels in AD roughly parallel the severity of dermatitis.

By classifying patients with AD into three groups on basis of a personal or a family history of ARD, the present study further demonstrates that the magnitude of serum IgE elevation in AD is associated with both a personal and a family history of ARD. Serum IgE values were slightly or moderately elevated in severe cases of "pure" AD who had neither a personal nor a family history of ARD, while very high serum IgE levels were consistently seen in severe cases of AD who had a personal history of ARD. These findings suggest that AD *per se* can bring about only a moderate elevation of serum IgE, and that the serum IgE elevation induced by AD is greatly amplified in AD patients who have a personal history of ARD.

Very high serum IgE values were observed in many patients with severe AD who did not have a personal history of ARD, but had a family history of ARD. It is likely that the AD-induced serum IgE elevation is also amplified in AD patients who have subclinical ARD or predisposition for ARD. On the other hand, a normal or moderately elevated serum IgE occurred in some cases of severe AD who had only a family history of ARD. This suggests that the AD group with only family history of ARD includes some patients who do not have ARD predisposition. It is known that atopic diseases are inherited with incomplete penetrance (16, 17).

Thus, on basis of the serum IgE producing potential, patients with AD may be classified into two subgroups: 1) those with ARD predisposition who have an enhanced ability for production of IgE, and 2) those without ARD predisposition who do not have an enhanced IgE producing potential (Fig. 5).

Hanifin and Rajka (18) have stated that serum IgE level greater than 2000 U/ml adds considerable support to the diagnosis of AD. But the present results show that such very high serum IgE levels may occur exclusively in those patients with AD who have a personal or family history of ARD, i.e., predisposition for ARD. Furthermore, it became evident that in patients with severe AD, serum IgE level of 2000 U/ml is a value which clearly separates patients with ARD predisposition from patients without such predisposition.

Finally, the occurrence of very high serum IgE values in approximately 60% of severe AD patients in the present study suggests that the remaining 40% of the severe AD patients did not have ARD predisposition. Interestingly, the distribution of AD patients with a personal ARD history and those with only a family ARD history were almost the same in the severe AD group and the mild AD group, suggesting that prevalence of coexistent ARD predisposition in AD patients has no relation to the severity of the dermatitis. From both these sets of data, it may be concluded that AD patients without ARD predisposition comprise about 40% of the total AD patients in a Japanese population.

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Acta Derm Venereol (Stockh) 1989; Suppl 144: 83–87

IgE Antibody to Sweat in Atopic Dermatitis

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Of 45 patients with atopic dermatitis skin-tested with their own sweat, 43 showed positive immediate-type skin reactions to titres between 1 and 256. Of 22 non-atopic patients 18 showed negative reactions. Skin reactivity of the atopic patients to the sweat and house dust did not run parallel. Radioallergosorbent test (RAST) using the sweat collected from a healthy subject detected IgE antibody in 24 atopic patients with a score from 0.5 to 3.5, whereas all the control subjects showed the score 0. This IgE antibody to sweat did not cross-react with the mite extract (Dermatophagoides farinae) or Staphylococcus aureus. These results indicate that atopic patients have specific IgE antibody to sweat. ***Key words: Sweat; IgE antibody; Atopic dermatitis.***

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It is known that patients with atopic dermatitis complain of itching during or after sweating. This phenomenon is one of the diagnostic features of atopic dermatitis (1). In addition, typical eczematous lesions of atopic dermatitis are often observed in the neck, the cubital and popliteal fossae and the other flexures which are the areas of sweat accumulation. It is clinically apparent that sweat has a significant role in the pathogenesis of atopic dermatitis. Sulzberger et al. (2) demonstrated that human sweat could produce whealing and itching when it got into cutaneous tissue, and the reaction was greater in atopic than in non-atopic individuals. They suggested that autologous sweat which was forced into the skin could produce itching in atopic dermatitis. However, the relation between atopic dermatitis and sweat is not fully understood. We investigated type I allergy to sweat in atopic dermatitis, and found that the patients with atopic dermatitis had specific IgE antibody to sweat in the serum.

MATERIALS AND METHODS

Patients

A total of 45 patients with atopic dermatitis (24 males and 21 females, mean age 21.7 years, range 6–50 years) were selected for this study. The diagnosis of atopic dermatitis was based on typical clinical features according to the diagnostic criteria of Hanifin & Rajka (1). Total serum IgE was measured by Phadebas radioimmunosorbent test (RIST) and were expressed as international units per millimeter (IU/ml).

As controls we examined 22 subjects (13 males and 9 females, mean age 25.9 years, range 6–52 years) without atopic history, consisting of 3 normals, 8 with urticaria, 3 with acne vulgaris. 8 with local skin infections.

Preparation of the sweat antigen

The patients with apparent eczematous changes or scratch marks on the back were excluded from the test. Sweat was collected by the "anaerobic" method described by Boysen et al. (3). The patient came to the hospital after taking a bath at home. An approximately 25×30 cm^2 area of the back of each patient was covered with vaseline and wrapped by Saran Wrap, and then the subject was warmed in a small sauna at 45–50°C. About 50–80 ml of clear sweat could be collected within an hour. The collected sweat was immediately sterilized using a Millipore filter and was kept frozen at −80°C until used. To differentiate an allergic reaction from an irritant reaction in skin test, the sweat was dialyzed against phosphate buffered (0.005 M, pH 7.2) saline (PBS) using a Visking tube. Protein concentration of the sweat was estimated by the method of Lowry et al. (4) using bovine serum albumin as standard and was shown to be approximately 0.4 mg/ml before dialysis and approximately 0.2 mg/ml after dialysis against PBS.

Skin test

Sweat from each patient was made in two-fold serial dilutions with saline and the series of the diluted sweat was injected into the normal-appearing skin of the patient's own forearm. A commercially available house dust allergen (Torii Co. 1:1000) was similarly diluted and injected in the opposite forearm. The dialyzed sweat was skin-tested in comparison to the undialyzed sweat in the same way. All subjects stopped taking oral antihistamine for 2–3 days prior to skin testing. The reading of the reaction was done 15 min after injection. A wheal greater than 9×9 mm or erythema greater than 20×20 mm was interpreted as positive. Skin test threshold was expressed as the maximum titre with positive reaction.

Radioallergosorbent test (RAST)

A large amount of sweat was collected from one of us (J. A.) who was healthy and non-atopic. The collected sweat was processed as described and freeze-dried, then solubilized with distilled water, and finally concentrated 2, 10 AND 50 times. These were coupled with cyanogen bromide-activated paper discs at 4°C for 3 days. Part of the 50 times-concentrated sweat was dialyzed against physiological saline. IgE antibody to sweat in the serum was measured using these sweat-coupled discs and Pharmacia RAST kit. The maximum value

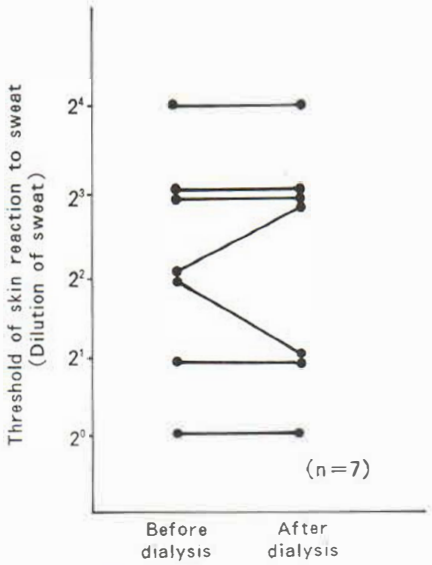

Fig. 1. Comparison of the antigenic activity of the sweat before and after dialysis.

obtained by four antigen preparations was regarded as the IgE antibody titer of the patient.

RAST-inhibition test

To examine cross-reactivity of the antibody to sweat, RAST inhibition test was performed using the mite extract and Staphylococcus aureus in a case who showed the RAST score (2.0). Since proper amounts of these two antigens to use for antibody absorption were not known, the assumption was made that 0.1 mg of the mite extract (Dermatophagoides farinae) or formalin-fixed Staphylococcus aureus (protein A negative Wood 46 strain) was equivalent to 1 mg sweat protein. The sweat was prepared in 1000, 100, 10, 1 and 0.1 μg protein/ml saline, and the mite extract and Staphylococcus aureus were prepared in 100, 10, 1, 0.1 and 0.01 μg weight/ml saline, respectively. Aliquots of the serum (0.08 ml each) were incubated at 37°C for 30 min with the same volumes of the five concentrations of the three antigens mentioned above in parallell with the same volumes of saline solution as the control. After incubation IgE antibody to sweat in each sample was measured by RAST using the discs coupled with 50 times concentrated and dialyzed sweat.

RESULTS

Skin test thresholds to own sweat in the patients with atopic dermatitis and in the normal controls

Forty-three of 45 atopic patients showed positive immediate-type skin reactions to sweat at titres between 1 and 256 (geographic mean of 43 positives; titre 11.8), whereas only 4 of 22 control subjects (3 with urticaria and 1 with folliculitis) showed positive reaction at titres between 2 and 8 (geographic mean of 4 positive cases; titre 6. This difference is statistically significant (chi square test, $p<0.01$). All subjects injected with sweat complained of piercing sensation during infusion of the sweat into the skin at the sweat concentration between titres 1 and 4. The sensation stopped immediately after infusion.

The effect of dialysis on the antigenic activity of the sweat

The antigenic activity of the sweat before and after dialysis as demonstrated by skin test thresholds showed no difference when examined in 7 patients (Fig. 1).

Comparison of the skin reactivities of the patients with atopic dermatitis to sweat and house dust

Fig. 2 demonstrates the relation between the skin reactivities to sweat and house dust of 45 patients with atopic dermatitis. The skin test thresholds to house dust were titres 1 000 to 64000 (mean of 36 positive cases; titre 8 300). The skin test thresholds to the two antigens did not run parallel ($r=0.2247$).

The relation of the serum IgE levels and the skin test thresholds to sweat in the patients with atopic dermatitis

Fig. 3 shows the relation between geographically plotted serum IgE level and the skin test threshold to

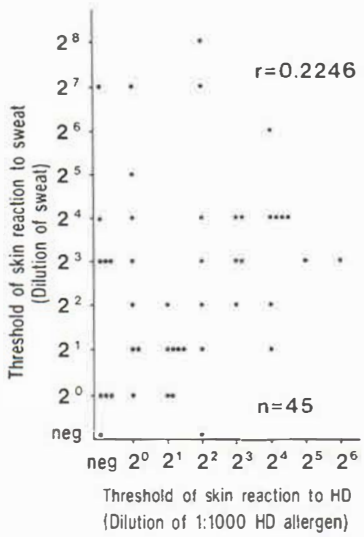

Fig. 2. The relation between the skin test reactivities to sweat and house dust in atopic dermatitis. No significant correlation was observed between the two.

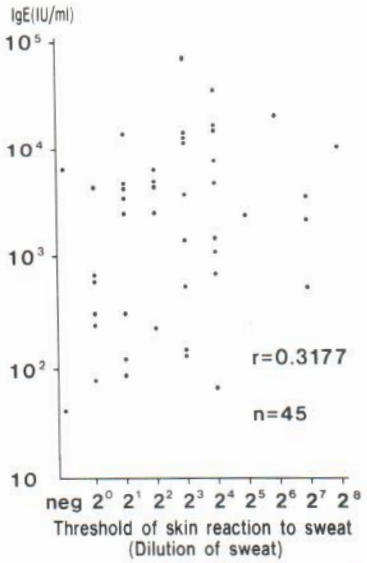

Fig. 3. The relation between serum IgE levels and the skin test thresholds to sweat in atopic dermatitis.

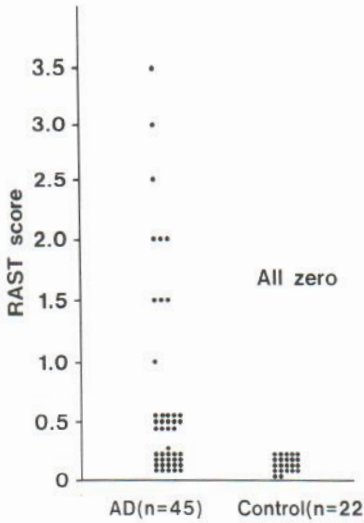

Fig. 5. IgE antibody titers to the sweat in patients with atopic dermatitis and controls.

sweat in each of 45 patients with atopic dermatitis. A statistically significant correlation was detected between the two ($r=0.3177$, $p<0.05$).

IgE antibody to sweat

Various RAST scores were obtained in the same serum sample by using different preparations of sweat antigens. Generally the higher the antigen concentrations the higher the scores and also dialyzed antigen gave higher scores (Fig. 4). Ten patients showed RAST scores from 1.0 to 3.5, 14 gave the score 0.5 and 21 gave the score 0. All 22 control subjects showed RAST score 0. The distribution of RAST scores shown in Fig. 5 seems to indicate that the RAST score 1.0 or higher may be a reliable marker for the presence of IgE antibody to the sweat. The data in Fig. 6 demonstrate no correlation in atopic dermatitis

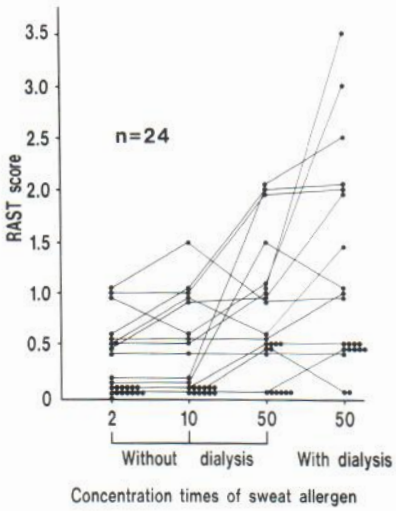

Fig. 4. RAST scores to the sweat detected by different sweat concentrations coupled to the disc.

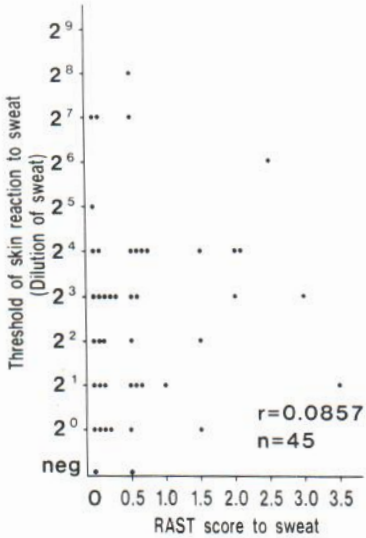

Fig. 6. The relation between the RAST scores to the sweat and the skin test thresholds to the sweat in patients with atopic dermatitis. No significant correlation was observed between the two.

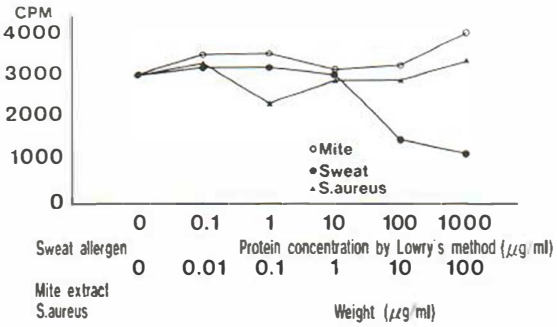

Fig. 7. The results of RAST-inhibition test. Mite extract and S. aureus did not reduce the conjugation of IgE antibody to the disc coupled with sweat.

between the skin test thresholds and the RAST scores ($r=0.0857$).

Specificity of the antibody to sweat

Amounts of IgE antibody to sweat were decreased by adding sweat to the serum in a dose dependent fashion, whereas they were not changed by adding the mite extract or Staphylococcus aureus (Fig. 7).

DISCUSSION

It was in 1953 that Sulzberger et al. (2) demonstrated that the sweat of the patients with atopic dermatitis induced wheal and flare reactions when injected into the patients' own skin. He hypothesized that sweat retention often observed in the skin of atopic dermatitis (dry skin) might cause sweat leakage into the skin and give irritant effects to the skin (5). Since then the relation of atopic dermatitis and sweat has continued to be the subject of investigation. Cotton et al. (6) studied the sweat of normal and atopic subjects by physical and chemical methods but could not detect any differences between the two. Förström et al. (7) found that human sweat had higher IgE values in atopic patients than in normals. Wilkinson et al. (8) detected anti-sweat precipitins (IgG) in the serum of atopic dermatitis, but they were also detected in hospital staff and in many skin disorders. Up to now, no one has attempted to demonstrate IgE antibody to own sweat in any disease conditions, including atopic dermatitis. There are possibly two reasons for this. First, sweat was thought to have nonspecific irritant effects to the skin that might make the analysis of immunological reactions difficult. In this investigation a piercing sensation was actually experienced in all subjects during infusion of the sweat into the skin. However, sweat induced a typical wheal and flare reaction in some subjects and not in the others. Therefore, the type I allergic reaction to the sweat seems to be independent from the irritant effects of the sweat. Secondly, sweat is usually contaminated with many substances, such as horny cells, skin surface bacteria and contactants (house dust and mites). To minimize contaminations with impure substances, we adopted Boysen et al. 's "anaerobic" method for sweat collection which is considered to be the only available method at present.

However, there is still a possibility that it may be contaminated with other antigenic substances. Patients with atopic dermatitis are often allergic to house dust (9, 10), mite (11), human dander (12, 13), or Staphylococcus aureus (14, 15), and therefore the antibody to sweat must be shown not to cross-react with these antigenic substances. Moreover, the patients with atopic dermatitis are colonized with Staphylococcus aureus on both involved and uninvolved skin (14). We compared the skin reactivities of atopic patients to the sweat and the house dust, but found statistically insignificant correlation between the two. Therefore, we do not think that sweat has serious cross-reactivity with house dust. We used RAST-inhibition test to examine cross-reactivity with mite and Staphylococcus aureus, and no cross-reactivity was demonstrated. Only the test with human dander was left. Although Berrens & Guikers (16) demonstrated that atopic dermatitis had IgE antibody to human dander, it is a complex mixture of cornified epidermal cells, sebum, sweat and numerous microorganisms. Cross-reactivity between sweat and human dander has been reported (17), but Sulzberger et al. (2) observed that the skin test with scale extract showed different skin reactions from sweat. Silpananta & Wilkinson (18) described that the characterization of main antigenic components of sweat differed from that of the human dander. From these data and since we did not know a method to collect pure dander that did not contain sweat, we omitted examination of cross-reactivity between sweat and dander. The sweat obtained by Boysen et al. 's method contained practically no horny materials. We believe that the results of our investigation can be regarded as evidence for the presence of IgE antibody to sweat. The incidence of positive IgE antibody to sweat (RAST score >0.5, 22.2%) was much less than positive skin tests (95.6%). However, this is similar to the results reported on mite allergy in chronic urticaria by Yamamoto et al. (19), who showed that histamine

release from leukocytes could be observed well before the detection of IgE antibody.

Skin test thresholds to sweat were correlated with serum total IgE levels in the atopic dermatitis patients we studied, Stone et al. (20) have shown a relation between various clinical data and IgE levels.

Major proteins of normal human sweat are albumin and alpha 1-antitrypsin (21), and more than 400 polypeptide components (22). Analysis of sweat antigens is left for future investigations.

ACKNOWLEDGMENT

We thank Mr T. Nakasuji for his collaboration in RAST.

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IgG Subclass Antibodies to Dietary Antigens in Atopic Dermatitis

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The role of IgG subclasses and IgG subclass antibodies in atopic disease is controversial. Serum IgG and IgG subclass (IgG1-4) antibodies to the two dietary antigens ovalbumin (OA) and β-lactoglobulin (BLG) were measured with ELISA-methods in 16 patients with mild or moderate atopic dermatitis (AD) and healthy controls. The IgG antibodies were measured in 31 patients with previous AD and controls. The IgG subclass antibodies to OA and BLG showed predominance of IgG4 and IgG1 for both patients and controls. The levels of IgG and IgG subclass antibodies to OA did not differ between the groups, but the levels of IgG and IgG4 anti-BLG antibody were higher in patients with active AD than in controls. The antibody levels did not correlate with severity of disease or with a history of food allergy/intolerance. IgG4 antibodies to dietary antigens may be elevated in AD, but the diagnostic significance of IgG subclass antibody measurement is limited.

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Interest in the IgG subclass antibodies to dietary antigens comes from the putative significance of IgG antibodies as "short term anaphylactic IgG antibodies" (1, 2) or as allergy modifying "blocking antibodies" (3). The present paper summarizes our results on IgG and IgG subclass antibodies to dietary antigens in patients with atopic dermatitis (4, 5). A brief description of the properties of the IgG subclasses, in particular IgG4, is given. The methods used are presented together with the results, and possible implications are discussed.

BACKGROUND

The four IgG subclasses are physicochemically characterized by differences in their polypeptide heavy chains, in particular in the so-called hinge region (6). Functionally, the IgG1 and IgG3 subclasses show a strong ability to activate the complement system and of binding to cellular receptors, whereas the IgG2 and IgG4 subclasses show weak activities in these respects (7, 8). As an exception, IgG4 exhibits binding to receptors on human basophils and may induce histamine release (9, 10). The latter finding, however, was not confirmed, as IgG4-antigen complexes failed to induce histamine-release from human leukocytes (11). A comparison of the properties of IgE and IgG4 is given in Table I. A shift from IgG1 to IgG4 of antivenom antibodies was observed in healthy bee-keepers who were repetitively stung (12) suggesting that IgG4 antibodies occur as a normal consequence of chronic antigen exposure.

It is well known that IgE levels are increased in atopic dermatitis (13). Serum levels of IgG4 were observed to be increased in adult patients with asthma or atopic dermatitis (14, 15, 16). In a study of children with AD raised IgG4 levels were found only in patients with concurrent asthma (17). The presence of IgG4 antibodies to grass pollen, house dust mite and food antigens were observed in asthma patients (18, 19). However, other studies showed that IgG4 antibodies to dietary antigens may occur in a considerable proportion of healthy subjects (20), and semiquantitatively determined levels of IgG4 antibodies to cow's milk did not relate to clinical cow's milk allergy in children (21). Thus, the role of IgG4 antibodies in atopic eczema is at present controversial.

PATIENTS

The patients studied by us form part of a previously published genetic study of atopic dermatitis in the general population (22). The patients (Table II) comprised 10 subjects with mild and 6 patients with moderate atopic dermatitis (AD), and 31 patients with a history of AD (only tested for total IgG antibodies). Three of the patients, two with a history of AD and one with moderate AD had experienced worsening of their eczema after the ingestion of food, but none reacted after the intake of milk or egg. Serum samples from the AD-patients were tested in parallel with a similar number of age- and sex-matched controls.

METHODS

IgG antibodies to ovalbumin (OA) from hen's egg and beta-lactoglobulin (BLG) from cow's milk were measured by en-

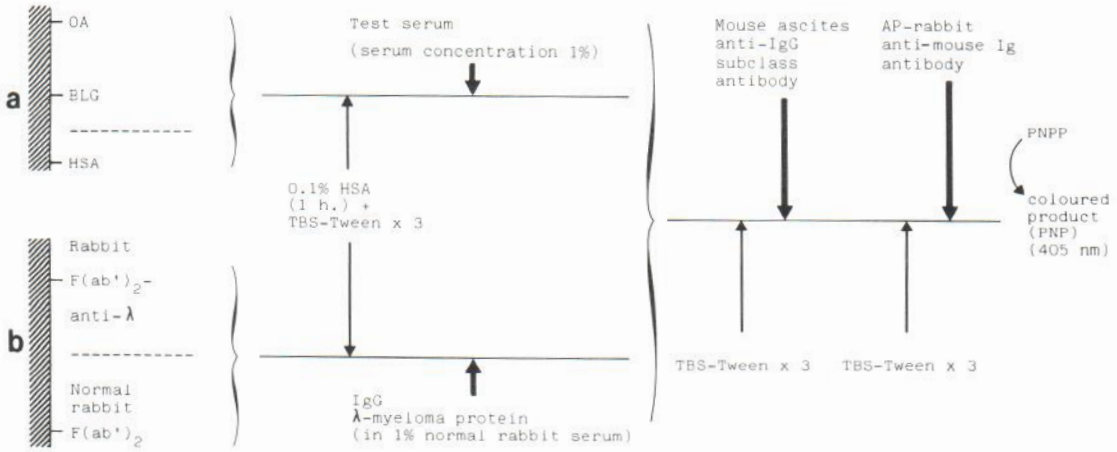

Fig. 1. Schematic drawing of the assay system for the determination of IgG subclass antibodies to dietary antigens, (*a*) test sera, (*b*) standards. From J Immunol Methods 1985; 83: 321, with the permission of the publishers (Elsevier).

zyme linked immunoassays (ELISA), as described previously (4). The assays were performed in microplates and included a biotin-avidin amplification step. The results were expressed quantitatively in mU/ml by referral to serial dilutions of a reference high-titered human serum.

For the measurement of IgG subclass antibodies to dietary antigens we employed ELISAs also developed by us (20). The principle for the IgG subclass antibody assays are shown in Fig. 1. Serum antibodies were bound to antigen (OA or BLG) on the solid phase, followed by the incubation with monoclonal anti-IgG subclass antibodies. Alkaline phosphatase-labelled rabbit anti-mouse Ig antibody was added and after further washings and the incubation with substrate the resulting colour reaction was determined in a photometer. To obtain an estimation of the antibody concentration the antibody binding (photometric measurement) was referred to a

Table I. *Biological properties of IgE and IgG4*

	IgE	IgG4
Serum concentration	0.1 μg/ml	100 μg/ml
Passage across placenta	–	+
Basophil/mast cell binding	+	+
Mediator release with		
(*a*) Specific anti-IgG4	–	+
(*b*) Specific antigen	+	–
Complement fixation	–	–
Genetic variants	+	+

Table II. *The characteristics of patients and controls*

	Mild atopic dermatitis ($n=10$)	Moderate atopic dermatitis ($n=6$)	Controls ($n=16$)
Respiratory atopy	1	3	0
Disease extent (0–16)			
Median	3	5	0
Range	2–3	5–10	0
IgE (μg/ml)			
Median	65	97	19
90% percentile	1 080	1 440	82

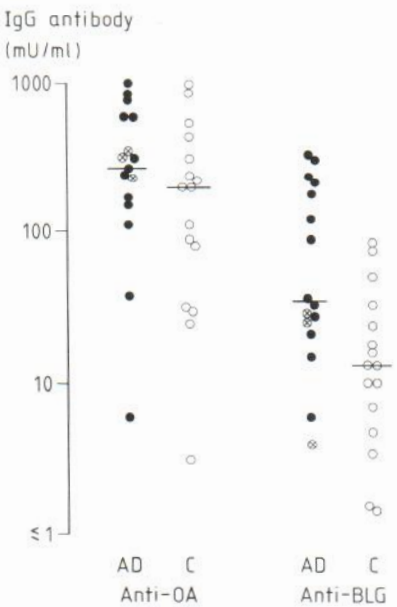

Fig. 2. Serum IgG antibodies to OA and BLG in patients with mild or moderate AD (●) and in controls (○). The logarithmic ordinate scale is expressed in arbitrary units. AD-patients with concomitant asthma/rhinitis are denoted as (⊗). Bars denote median values. From Allergy 1986; 41: 379, with the permission of the publishers (Munksgaard).

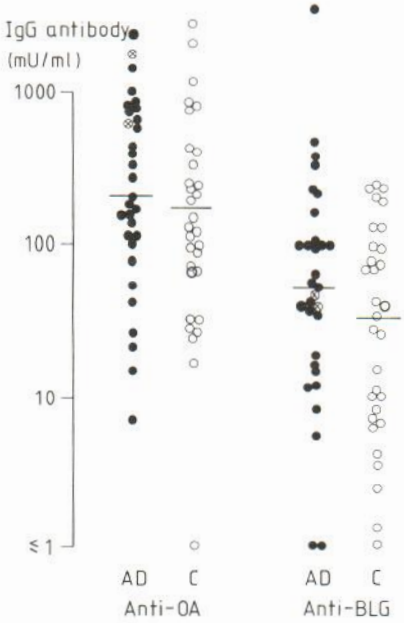

Fig. 3. Serum IgG antibodies to OA and BLG in patients with a history of AD (●) and in the corresponding controls (○). AD-patients with asthma/rhinitis (⊗). Bars indicate median values. From Allergy 1986; 41: 379, with the permission of the publishers (Munksgaard).

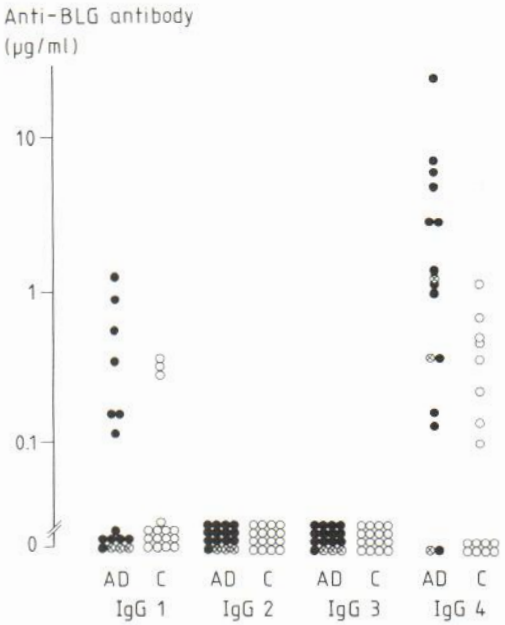

Fig. 5. IgG subclass antibodies to BLG in patients with AD (●) and in controls (○). AD-patients with concomitant asthma/rhinitis (⊗). From Allergy 1986; 41: 386, with the permission of the publishers (Munksgaard).

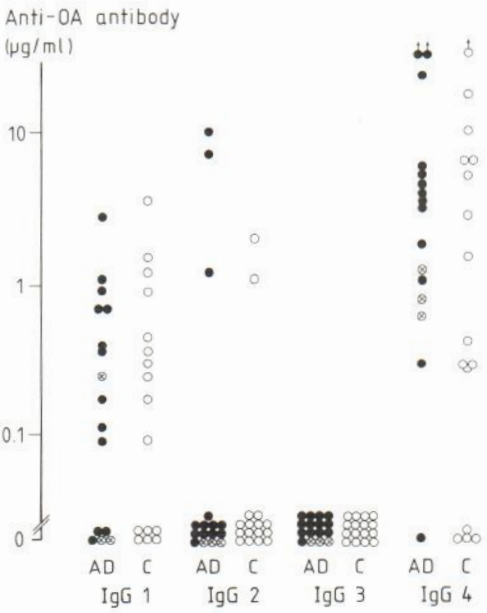

Fig. 4. IgG subclass antibodies to OA in patients with AD (●) and in controls (○). AD-patients with concomitant asthma/rhinitis are denoted by (⊗). From Allergy 1986; 41: 386, with the permission of the publishers (Munksgaard).

standard curve with established IgG subclass myeloma protein.

The statistical evaluations were made with the nonparametrical Mann-Whitney U-test as a two-tailed test.

RESULTS

The determination of IgG antibodies to OA and BLG showed detectable antibodies in the large majority of both patients with active AD and controls (Fig. 2) and the patients with previous AD and their controls (Fig. 3). The levels of IgG anti-OA antibodies did not differ between the groups, whereas the levels of IgG anti-BLG antibodies were significantly higher ($p < 0.05$) in patients with active disease than in their corresponding controls (Fig. 2). However, the anti-BLG antibody levels were not statistically different between the patients with active and the patients with previous AD.

The IgG subclasses of antibodies to OA were measurable in a high proportion of both patients and controls in IgG1 and IgG4, at comparable levels (Fig. 4). Also, a few serum samples contained antibody of the IgG2 subclass. Antibodies to BLG were observed only in IgG1 and IgG4 (Fig. 5). The IgG4 anti-BLG antibodies were significantly higher ($p < 0.001$) in AD-patients (median 1.1. µg/ml, range 0–24.0 µg/ml) than in controls (median 0.05 µg/ml, range 0–1.1 µg/ml).

DISCUSSION

From the present studies we may conclude that IgG antibodies to OA and BLG are produced in the majority of AD patients and in normals as well. This antibody activity is localized mainly in the IgG4 subclass, although an absolute subclass restriction is not present. The levels of IgG4 anti-BLG antibodies were significantly higher in atopic dermatitis patients than in controls. However, a considerable overlap was observed between the patients and the controls, so the determination of IgG4 anti-BLG antibodies seems of limited diagnostic value.

Our results of antibody measurements, which comprise all four subclasses, are partially in concordance with other studies of IgG4 antibodies alone. Merrett et al. (16) observed in AD patients high levels of IgG4 antibodies to a number of foods, including egg white, milk, codfish and peanut. Shakib et al. (23) found no difference in IgG4 antibodies to purified milk and egg antigens between sera from AD patients and normals, but the majority of both patients and controls had undetectable levels of antibody to e.g. BLG. Rowntree et al. (24) measured IgG4 antibodies to OA and BLG in adult AD-patients and observed the frequent occurrence of IgG4 anti-OA antibody as a significant proportion of total IgG anti-OA antibody. Few patients and controls had IG4 anti-BLG antibody, with no difference between patients and controls. In a prospective study (24) of children from atopic families the proportion of IgG4 to OA and BLG increased up to 5 years of age. Higher IgG4 anti-OA antibody levels were observed in 3-year-old children with positive prick test or IgE antibody (RAST) to OA, but no significance-testing was performed on these data.

Only three of the patients in our study were suspected of clinical food allergy as evaluated from their history and skin prick tests (data not shown). These patients did not show particularly high IgG4 antibody levels. However, we did not perform regular food diet and provocation tests in this study, leaving open the theoretical possibility that unrevealed milk or egg allergy could influence the antibody levels. Studies on IgG subclass antibodies specifically in relation to milk allergy are in progress. Furthermore, studies of patients with severe AD may show more clear results than the present, population-based patient material.

Genetic factors may influence both the antibody levels and the disease AD (22). In healthy twin subjects we demonstrated about one third of genetic dependence of IgG anti-OA and anti-BLG antibody levels (25). The levels of IgG4 antibodies were in a recent report related to Gm allotypic markers (26). However, we did not find any relation between the levels of antibodies to OA and BLG and the HLA-A, B, C antigens or the Gm and Km allotypes (Husby et al., unpublished). As to AD, no association was observed between the disease and the HLA-A, B or C antigens and several other genetic markers including the Gm and Km allotypes (27).

The biological and clinical significance of IgG4-antibodies is at present unclear, as noted above. Clearly, more research is needed to distinguish the physiological function of IgG4 from its putative role in atopy as a reaginic antibody or a "blocking" antibody. Further characterization of the IgG4 receptor on human basophils and mast cells is awaited.

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Acta Derm Venereol (Stockh) 1989; Suppl 144: 93–96

Immunohistochemical Studies on Dust Mite Antigen in Positive Reaction Site of Patch Test

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We demonstrated that eczematous reactions could be induced by patch testing with mite antigens in the majority of patients with atopic dermatitis (AD). By using immuno-double labelling technique, many mite antigen-bearing Langerhans cells were seen in the epidermis in the early stage of the patch testing. Twenty-four hours later, these cells were observed only in the deep dermis. Immunoelectron microscopically, it was found that the mite antigens were trapped by macrophages, which were apposing lymphocytes.

On the other hand, we observed that Langerhans cells and Leu 3a positive cells in the AD lesions carried IgE molecules. Furthermore, many IgE-positive dendritic cells bearing mite antigen were seen in the positive patch testing sites.

Taken together, IgE-mediated contact hypersensitivity to mite antigen may play an important role in the pathogenesis of AD. ***Key words: Mite antigen; IgE, Langerhans cells; T-lymphocytes.***

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Many patients with atopic dermatitis (AD) show an immediate reaction to mite allergen in skin tests. Moreover, their serum IgE levels are usually high, and IgE-RAST for mite antigens are frequently positive. We previously reported that serum levels of mite-specific IgG, IgG4, IgE and IgE immune complexes in patients with AD were significantly higher than those in healthy controls. However, the skin lesions of AD are characteristic of delayed-type hypersensitivity, differing from the immediate wheal reaction. To investigate if the two different immune reactions play a role in the pathogenesis of AD, patch testing with mite antigens, along with immunohistochemical and immunoelectron microscopic studies, were done.

MATERIALS AND METHODS

Patients and control subjects

The following groups were investigated: Twenty patients with AD (aged 2–25 years, mean 14.9 years; serum IgE level 20–12 306 U/ml, mean 1 858.9 U/ml) and twenty healthy volunteers having no history of atopy (aged 21–25 years, mean 22.5 years; serum IgE level 20–297 U/ml, mean 107.1 U/ml). All subjects gave their informed consent.

Antigens. Antigen solutions of Dermatophagoides pteronyssinus (DP) and D. farinae (DF) were prepared from full-grown mite cultures using the method of Miyamoto et al. (1). Before solutions were extracted, the source culture was defatted in anhydrous acetone and homogenized with phosphate buffered saline (0.005 M phosphate buffer pH 7.2 containing 0.15 M NaCl). After centrifugation at 15 000 g for 20 min, the supernatants were dialyzed against distilled water and freeze-dried. The antigens obtained from whole cultures of DP and DF were denoted DP-WCE and DF-WCE respectively. Mite-free culture medium extract (CME) was also prepared using the same procedure.

Patch tests. White petrolatum containing 0.1 % sodium lauryl sulphate (SLS) was used as the vehicle in accordance with the SLS provocative patch test described by Kligman (2). Patch testing with 0.1 % (w/w) DP-WCE and 0.1 % (w/w) DF-WCE, in the vehicle was performed with Finn Chambers on clinically normal skin of the back. On control sites, vehicle alone and vehicle containing 0.1 % (w/w) mite-free CME were applied. The test reactions were read after 48 h and evaluated according to the criteria of ICDRG.

Histopathology. Five biopsy specimens were obtained from positive patch test sites of 5 patients. Moreover, to investigate the time-course of the inflammatory reaction of the patch test site biopsies were taken after 1 h, 6 h, 24 h and 48 h. Furthermore, twelve biopsy specimens were obtained from active lesions of the other AD patients group, in order to compare the reaction with that of positive patch test sites. One half of each biopsy specimen was prepared for routine histological examination, while the remaining half was processed for immunohistochemical and immunoelectron microscopical studies as described below.

Antisera and affinity purified antibodies. Antisera to DP-WCE and DF-WCE were obtained by immunizing New Zealand white rabbits with both antigens using complete Freund's adjuvant.

Each antiserum was absorbed with CME. For specific purification, 10 mg of each extract was bound to Sepharose activated with CNBr. One hundred ml of anti-DP-WCE or anti-DF-WCE antisera was passed over an affinity column containing DP-WCE or DF-WCE immunosorbent. The bound antibody was eluted with 0.1 M glycine-HCl, pH 2.5, dialyzed with PBS, and stored at −20°C and labelled affinity purified anti-DP (1 mg/ml) and anti-DF (1 mg/ml) antibodies. By immunodiffusion, this anti-mite antibody showed several cross-reactive precipitation lines against DP-WCE, DF-WCE and house dust extract. But reactions to CME, whole culture extracts of Tyrophagus putrescetiae and Glycyphagus priva-

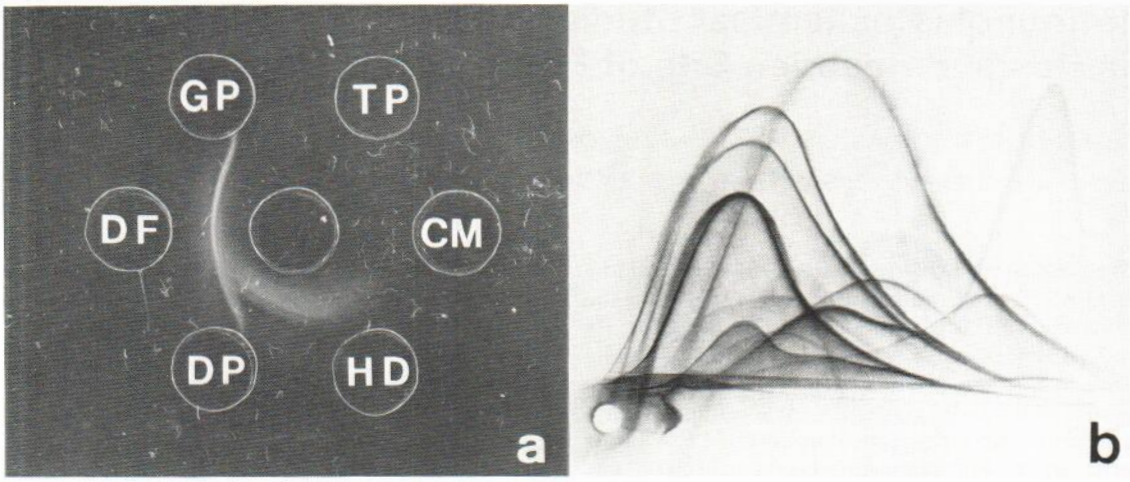

Fig. 1. Immunodiffusion and crossed immunoelectrophoresis analysis of anti-mite antibody (mixture of affinity purified rabbit anti-DP and anti-DF antibody). (*a*) Immunodiffusion against extracts of DF, DP, house dust (HD), mite-free culture medium (CM), Tyrophagus putrescetiae (TP) and Glycyphagus privatus (GP). The center well contained anti-mite antibody. (*b*) Crossed immunoelectrophoresis pattern of DF-WCE reacting with anti-mite antibody. 100 μg applied in well. Anodical antibody-containing gel with 5 μl/cm^2. Electrophoretic conditions: 1) dimension 10 v/cm, for 45 min. 2) dimension 2 v/cm for 16 h. Stained with Coomassie Brilliant Blue.

tus were not observed (Fig. 1 *a*). Crossed immunoelectrophoresis demonstrated up to 28 precipitates for the DF-WCE (Fig. 1 *b*) and 27 for the DP-WCE respectively.

Processing of skin biopsies. Biopsy specimens were fixed using 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 6 h at 4°C for immuno-double labelling and immunoelectron microscopy. They were then washed in 10–20% sucrose in PBS overnight, snap-frozen and stored at −80°C. The 5 μm frozen sections were incubated with the anti-mite antibody (1:50 in PBS) for 30 min at 37°C, followed by FITC conjugated anti-rabbit IgG (TAGO, 1:50 in PBS) for 30 min at 37°C. Afterwards, the sections were treated with monoclonal OKT 6 or Leu 3a (1:100 in PBS) for 30 min at 37°C, followed by incubation with rhodamine conjugated anti-mouse IgG (TAGO, 1:100 in PBS) for 30 min at 37°C. The specimens were then examined under a Zeiss fluorescent microscope with an appropriate FITC and rhodamine filter setting.

RESULTS

Results of the patch tests are shown in Table I. Fourteen of twenty patients with AD showed positive reactions to DP-WCE and thirteen were positive to DF-WCE. In the mite RAST-positive AD group (a RAST score of 2 or more was regarded as positive), the positive reaction rate of patch test was higher than in the mite RAST negative patient group. No positive reactions were observed at the control sites of the patients or the healthy volunteers. The positive reaction sites showed edematous erythema with papules and vesicles. Histologically, acanthosis, spongiosis and perivascular lymphocytic infiltration was observed. These reactions resembled AD lesions clinically and histologically. By using immuno-double labelling technique, it was demonstrated that mite antigens were present in the epidermis and the dermis, mostly located to the OKT 6 positive cells (Langerhans cell, LC). And it was observed that mite antigens were distributed around a cluster of Leu 3a positive cells in the dermis of a positive test site (Fig. 2).

The immunoelectron microscopic study revealed that some macrophages in the dermis exhibited positive labelling with antimite antibody on the cytoplasmic membrane as well as positively labelled small

Table I. *Results of patch tests with mite antigen in patients with atopic dermatitis (n=20)*

	Total no. of patients	Patch test with DP-WCE	
		Positive	Negative
RAST positive for DP	14	13	1
RAST negative for DP	6	1	5
RAST positive for DF	13	12	1
RAST negative for DF	7	1	6

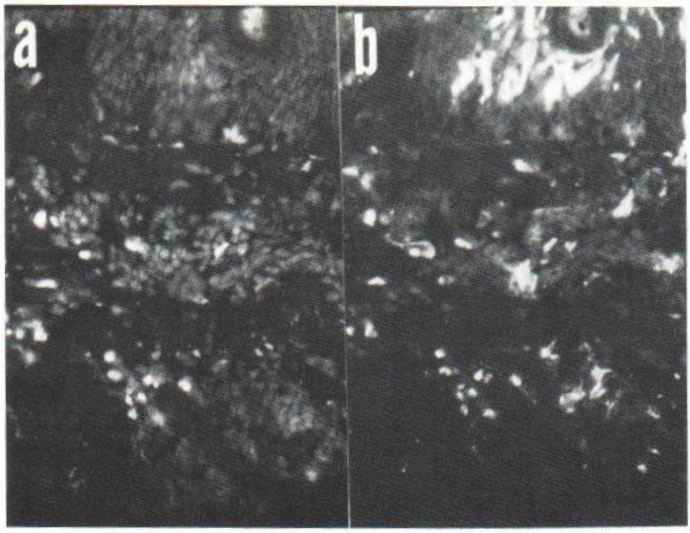

Fig. 2. The micrographs illustrate the immuno-double labelling findings. The biopsy was taken from a positive patch test site. Micrograph *a* reveals the presence of mite antigens by FITC immunofluorescence. Micrograph *b* shows rhodamine immunofluorescence of OKT 6 positive cells. It can be seen that many OKT 6 positive cells carry mite antigens.

phagocytosed particles in the cytoplasm (Fig. 3). These macrophages were often in apposition to lymphocytes.

Mite antigen bearing LCs were seen mainly in the epidermis from 1 to 6 h of the patch testing. After 24–48 h, many mite antigen-bearing LCs were observed in the dermis. Mite antigen bearing LCs were seen in the active lesions in 7 of 12 AD patients.

DISCUSSION

It has been reported by some authors (3–7) that inhalant allergens such as house dust mite could provoke delayed type skin reactions in patients with AD.

In this study, according to the modification of SLS provocative patch test by Kligman (2), an eczematous reaction could be induced by patch testing with mite antigen after 48 h in the majority of patients with AD. Mitchell et al. (4) and Gondo et al. (7) have succeeded in reproducing eczematous lesions in abraded skin of AD patients. Abrasion and SLS application may simulate naturally occurring conditions, scratching and sweating, in AD.

Our immunohistochemical study demonstrated that mite antigen invaded the skin and was trapped by LCs both in positive patch test sites and in the lesions of AD. And it can be hypothesized that LCs may trap mite antigens in the epidermis, migrate to the dermis, appose lymphocytes and present the antigen to these. Silberberg et al. (8) reported that after DNCB challenge in passiely sensitized guinea pigs, LCs in the epidermis decreased after 6 hrs and increased in the dermis, and apposition of mononuclear cells to LCs were seen mainly in the dermis at 3 or more h after challenge. Our results are compatible with theirs.

Recently, Bruynzeel-Koomen et al. (9) reported that IgE molecules were seen on epidermal LCs in AD lesions and this phenomenon seemed to be specific for patients with AD. We confirm this finding by use of the immuno-double labelling method. However, many IgE-carrying LCs were found not only in the epidermis but also in the dermis. Furthermore, IgE-

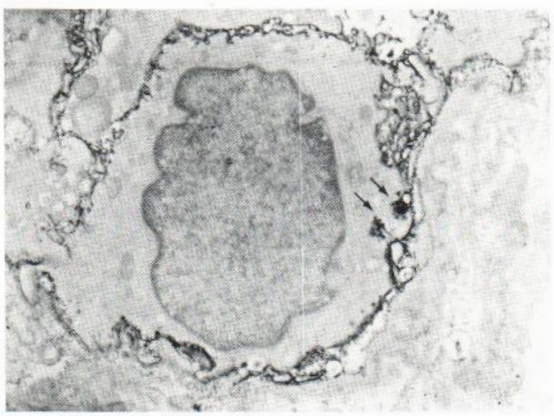

Fig. 3. Immunoelectron microscopic picture. The same biopsy specimen as shown in Fig. 1. A macrophage in the dermis of a patch-tested site carries the granular particles on the cell membrane. Some of the particles, which are mite antigens, are phagocytosed (arrow).

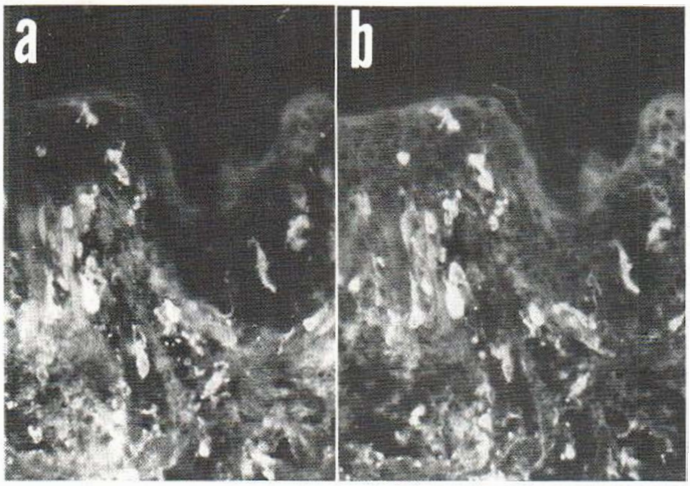

Fig. 4. Both micrographs illustrate the immuno-double labelling finding with IgE molecules and mite antigens. The specimen was taken from a positive reaction site of dust mite patch testing. Micrograph *a* shows FITC-immunofluorescense of IgE molecules. Micrograph *b* reveals rhodamine immunofluorescense of mite antigens. Many dendritic cells bearing both IgE molecules and mite antigens are observed.

carrying LCs were observed in the epidermis and dermis of the lesions of allergic contact dermatitis and other pruritic skin diseases, although less frequent than in AD. Recently, Lever et al. (10) demonstrated dermal IgE-bearing T lymphocytes in the dermis of AD lesions. The same findings were observed in the present study. Ishizaka et al. (11) reported that T lymphocytes bearing Fcε receptors could produce IgE binding factor and modulate the response to IgE. These IgE-carrying cells were also seen in the positive patch test sites and some of these cells were dendritic and carried mite antige (Fig. 4). It is still unclear whether antigen bind to IgE molecules on the surface of LCs. However, it may be hypothesized that IgE-mediated allergic contact sensitivity to mite allergen is playing an important role in the pathogenesis of AD.

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Altered Production of Immuno-modulating Cytokines in Patients with Atopic Dermatitis

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Atopic dermatitis (AD) represents an inflammatory skin disorder which is characterized by many signs of immunodeficiency. Particularly, decreased lymphoproliferative responses upon stimulation with mitogens as well as bacterial antigens were reported repeatedly. Since there is increasing evidence for a network of immuno-modulating cytokines playing a crucial role in the regulation of immunity and inflammation, in the present study we investigated whether an altered production of these mediators is one of the pathomechanisms responsible for the altered immune response in AD. For this purpose the 24-h supernatants of LPS- and PHA-stimulated or unstimulated mononuclear cells (MNC) from patients with AD of a moderate to severe disease activity and from nonatopic healthy controls were tested for Interleukin-1 (IL-1) and Interleukin-2 (IL-2) activity. Whereas supernatants of unstimulated MNC of AD patients and controls did not contain significantly different levels of these cytokines, LPS-stimulated MNC of AD patients released significantly less IL-1 in the supernatants. Similarly, the production of IL-2 by PHA-stimulated MNC of AD patients was significantly decreased in comparison to the controls. Moreover, there was a strong correlation between IL-1 and IL-2 levels. These findings indicate that diminished lymphoproliferative responses in AD may partly be caused by a decreased capacity of MNC to release immuno-modulating cytokines, even upon appropriate stimulation.

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Atopic dermatitis (AD) represents an inflammatory skin disorder which is characterized by significant changes in humoral and cell mediated immunity, particularly by alterations in T-cell-related functions (1–7). Clinically, these abnormalities are manifested as an enhanced susceptibility to severe infectious skin diseases, particularly viral infections with herpes simplex and vaccinia. Furthermore, an increased incidence of warts and molluscum contagiosum has been reported (1, 6). Since a network of cytokines including interferons, colony-stimulating factors and interleukins appear to regulate many effects of immunity and inflammation, it has been speculated that these patients have a defect in the capacity to produce interferons in response to viral antigens. However, the production of interferon-alpha and -gamma in whole blood cultures of patients with AD was found to be unchanged (6). In contrast, reduced lymphocyte responses to T cell mitogens or recall antigens in vitro, most evident during severe exacerbations of the disease, have been reported (1, 5–7). Therefore, the present study was performed to investigate whether one of the pathomechanisms responsible for these changes may be due to an altered production of cytokines.

MATERIALS AND METHODS

Patients and controls

The atopic dermatitis (AD) group consisted of 10 patients (1 male, 9 females) with moderate to severe disease activity: $\geq 25\%$ of body surface area was involved, all patients had excoriated skin lesions with intensive pruritus. The diagnosis was established according to the criteria of Hanifin and Rajka (8). The control group consisted of 7 healthy blood donors (6 males, 1 female). Atopy was excluded by history and laboratory findings. Neither control nor patients had received local or systemic steroid therapy, or therapy with ultraviolet light for at least 3 weeks prior to blood collection.

Isolation of mononuclear cells (MNC)

Unfractionated MNC were isolated from heparinized (50 units/ml) peripheral blood of patients and controls as described previously (9). MNC were adjusted at a concentration of 5×10^6/ml in serum-free HEPES-buffered Eagle's minimal essential medium (MEM) containing 50 µg/ml bovine serum albumin (BSA) and incubated in presence of 0.5 µg/ml lipopolysaccharide (LPS), 5 µg/ml PHA or without stimulus at 37°C in 95% humidified air. After 24 h supernatants were collected, centrifuged, sterile filtered and stored at −70°C until testing.

Bioassays

Supernatants were tested for Interleukin-1 (IL-1) activity using the thymocyte costimulator assay and the IL-1 mediated

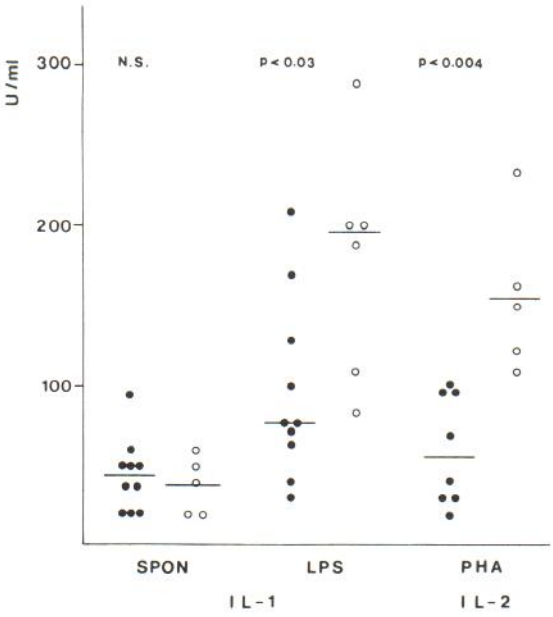

Fig. 1. Spontaneous (SPON) and LPS-stimulated (LPS) release of Interleukin-1 (IL-1) and PHA-stimulated release of Interleukin-2 (IL-2) by MNC of patients with atopic dermatitis (●) and non-atopic controls (○). Bars indicate medians.

proliferation of a murine T cell line (D10) as described previously (10, 11). IL-2 activity was evaluated measuring the proliferation of an IL-2 dependent cytotoxic mouse T-cell line (CTLL-16) as described (12). Results are expressed as U/ml which were calculated by comparing serial dilutions of samples with serial dilutions of a known IL-1 or IL-2 preparation containing 100 U/ml (10).

Reagents and stimuli

Phytohemagglutinin (PHA) was obtained from Wellcome (Burgwedel, FRG). S-form LPS from Salmonella abortus equi was a kind gift of Dr C. Galanos, MPI for Immunobiology, Freiburg, FRG. Purified BSA was from Sigma, Munich, FRG. Culture media were purchased from Biochrom-Seromed, Berlin, FRG.

Statistical analysis

Statistical evaluations were performed by using the Mann-Whitney U-test. For evaluation of correlations Spearman's rank correlation coefficient was calculated.

RESULTS

MNC of AD patients and controls released detectable amounts of IL-1 spontaneously (Fig. 1). However, no significant differences could be detected between patients and controls. In contrast, the levels of IL-1 activity in the supernatants of LPS-stimulated MNC were significantly decreased in AD patients (Fig. 1). Since thymocyte proliferation may be altered by other cytokines present in MNC supernatants IL-1 activity, additionally, was evaluated using the IL-1 sensitive murine T cell line D10 which does not proliferate in response to interleukin-6 and tumor necrosis factor. Using this bioassay similar levels of IL-1 activity were detected in the MNC supernatants tested. Moreover, a monoclonal antibody directed against IL-1 (13) blocked the thymocyte- or the D10 proliferation-inducing capacity of the MNC supernatants suggesting that IL-1, mainly, is responsible for the bioactivity measured (data not shown).

Similarly the release of IL-2 in the supernatants by PHA-stimulated MNC was significantly lower in patients with AD when compared with the control group (Fig. 1). There was no detectable production of IL-2 spontaneously as well as upon stimulation with LPS in patients and controls. Levels of IL-1 and IL-2 correlated significantly ($r=0.791$, $p \leqslant 0.004$).

DISCUSSION

Activation of T cells is dependent on the release of immunomodulating cytokines: Following binding of the T cell to the antigen-presenting cell the T cell stimulates the antigen-presenting cell to produce IL-1 by a mechanism which is not completely clarified at present. IL-1 in association with antigen stimulation induces IL-2 receptors on the T cells and stimulates T cells to release IL-2 which drives antigen activated cells into proliferation (14). Therefore, decreased lymphoproliferative responses could be the result of a diminished capacity of MNC to release IL-1 and IL-2 upon appropriate stimulation. Our data show that MNC from AD patients release significantly less IL-1 and IL-2 following stimulation whereas the basal IL-1 production was unchanged. The results are in agreement with a previous report of Räsänen et al. (15) who could show that purified monocytes of AD patients produced clearly less IL-1 in response to stimulation than monocytes from healthy controls. Therefore, the decreased production of IL-1 is apparently not due to suppressive factors derived from T-lymphocytes. These data support the hypothesis that in addition to T cell-related changes monocyte functions are impaired in AD. Depressed lymphoproliferative responses upon stimulation with T cell mitogens may be caused by diminished production of IL-2 upon stimulation. Our results clearly demonstrate that MNC of AD patients release significantly less IL-2 in

the supernatants following stimulation with the T cell mitogen PHA in comparison to healthy controls.

Levels of IL-1 and IL-2 were significantly correlated suggesting a general defect of MNC from AD patients to produce cytokines. However, production of interferons in whole blood cultures was recently shown to be unchanged in AD (6). Furthermore, serum IL-2 receptor levels were found to be significantly increased in AD (16). Increased serum levels of IL-2 receptor have been detected in different diseases accompanied with T cell activation and changes in the immune system. Therefore, it appears very unlikely that depressed lymphoproliferative responses in AD are the result of a basic defect of patients' MNC to produce immuno-modulating cytokines upon stimulation. We, therefore, suggest that the decreased production of cytokines by blood MNC is due to down-regulation induced by cytokines released from activated cells in the inflamed dermis. Alternatively, the hyporesponsiveness of MNC in vitro could be a sign of "exhaustion" following excessive stimulation in vivo. These suggestions are supported by the finding that altered lymphoproliferative responses normalize with clinical remissions (17).

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Absorption of Egg Antigens by the Gut Observed by Oral Prausnitz-Küstner (Walzer) Reaction in Atopic Dermatitis

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Sera from 50 children (27 boys and 23 girls, under the age of 3 years) with atopic dermatitis allergic or not to hen's egg shown by skin test or radioallergosorbent test (RAST) were passively transferred to recipients which were then challenged with injection of egg antigen (Prausnitz-Küstner (P-K) test) or with ingestion of a raw egg (oral P-K test). Thirty-one patients showed positive P-K reaction with serum titers from 2 to 8 192. Fifteen of the P-K positive cases were also positive in the oral P-K test with titers from 2 to 256. The ratio of the oral P-K titer and the P-K titer in each positive case was from 1 : 2 to 1 : 32. The results indicate that a high percentage of atopic dermatitis patients with egg allergy have IgE antibody in the serum capable of reacting with an ingested egg. ***Key words: Atopic dermatitis; Food allergy; Egg allergy; Prausnitz-Küstner reaction; Walzer reaction.***

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Infantile atopic dermatitis is often associated with food allergy. However, whether or not and to which extent these children and their breast feeding mothers should avoid allergic foods is controversial (1). Elimination diet in the children with atopic dermatitis was reported to be beneficial (2, 3) or of limited value (4, 5). There are so many questions to be solved before this problem is completely understood: Are the food antigen inactivated by the antibody secreted into the digestive tracts? How much and which part of the food antigen can be absorbed by the gut? Which immunoglobulin classes (or subclasses) of the antibody can react with the food antigen circulating in the blood stream and the skin, and what is the result of the reaction? In this paper we will try to answer the question; what kind of and which strength of reaction can occur between IgE antibody in the serum of patients with atopic dermatitis and the ingested food.

In 1921 Prausnitz & Küstner reported that food allergy could be passively transferred with the serum of one patient to another person (6). He induced the reaction by injecting foood extract into the skin where the serum was inoculated a day before. In 1926 Walzer noticed that a similar reaction could occur in the serum-inoculated skin by ingestion of the food antigens (7). He injected the recipient with serum from two children, one allergic to egg and the other to herring. Several hours later the recipients ate a raw egg or 50 g of herring. Blush, erythema and wheal appeared 10–20 min later with the egg serum and 30–100 min later with the fish serum. He thought this was a direct demonstration of absorption of incompletely digested food. We adopted these tests to evaluate the nature and the intensity of IgE egg allergy in the atopic children.

PATIENTS AND METHODS

Patients

Fifty children (27 boys and 23 girls) with atopic dermatitis under the age of 3 years (6 months or under; 12, 7 to 12 months; 19, 1 year; 26, and 2 years; 7) were studied. Allergic states of these children to hen's egg as determined by skin test to the whole egg allergen and IgE-radioallergosorbent (RAST) to egg white are described later. Thirty-two of the 50 patients were positive in the skin tests and 33 were positive in the RAST.

Skin test

A commercially available whole egg antigen (1 : 1 000, 0.025 ml. Torii Co.) was injected intracutaneously in the forearm of the patients and diameters of wheal and erythema were recorded at 15 min. A wheal greater than 9×9 mm or erythema greater than 20×20 mm were interpreted as a positive reaction.

RAST

Phadebas IgE-RAST kit to egg white was used and the results were shown as scores. RAST scores of 2.0 or greater were interpreted as positive.

Passive transfer (P-T) of the patient's serum

The conditions for performing this were; (1) the patient had been healthy and had no history of hepatitis, abnormal liver function tests or positive hepatitis B surface antigen, (2) a

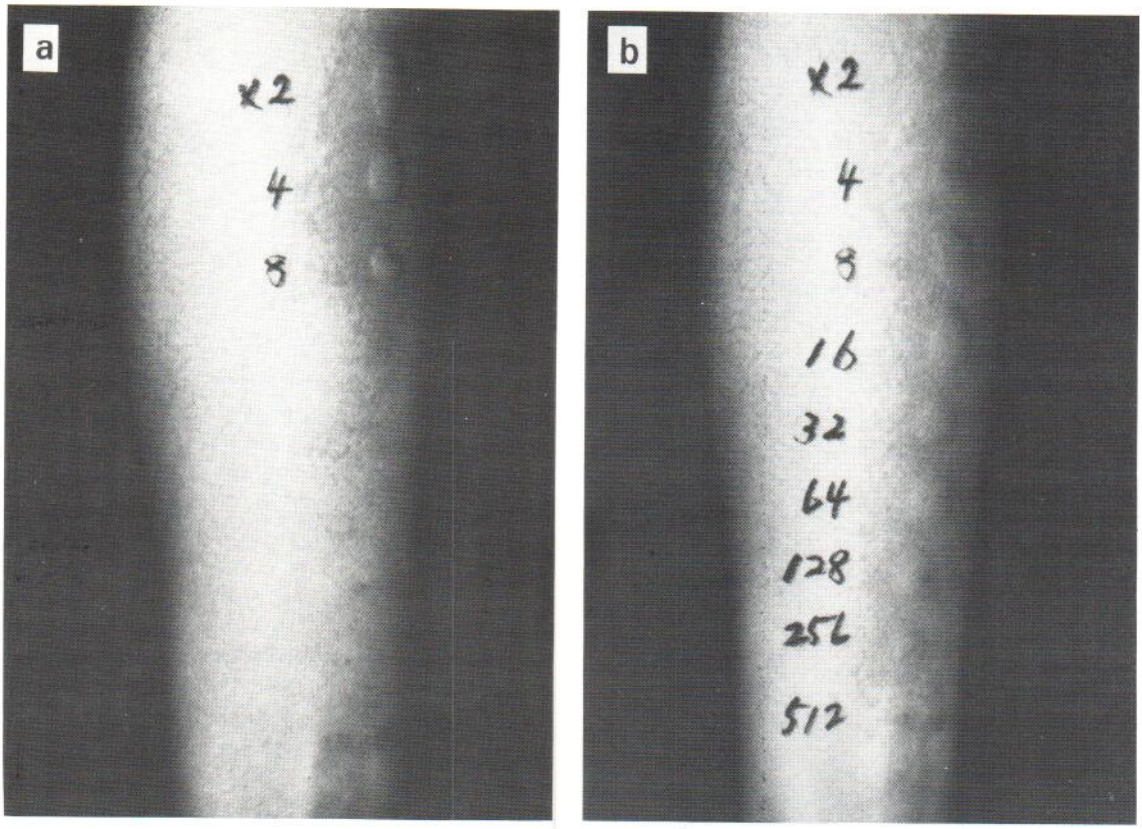


Fig. 1 a–c. Positive oral P-K reactions at 1, 2 and 4 h after a raw egg ingestion.

nonallergic recipient could be obtained from the parents who was well aware of the medical history of the patient, and (3) the consent was obtained from the recipient. Two-fold serial dilutions (routinely titers 2 to 1024) of the patients serum were injected intracutaneously in both forearms of the recipient. One arm was prepared for the Prausnitz-Küstner test and the other for the oral Prausnitz-Küstner test. Eggs and all kinds of egg containing food were forbidden to the recipients fromt he day prior to the passive serum transfer to the day of the P-K test.

Prausnitz-Küstner (P-K) tests

Two days after P-T of the serum, the same egg antigen as used for the skin testing was injected in the P-T sites and the reaction was read at 15 min. The maximum serum dilution that gave a positive reaction was recorded as the antibody titer of the serum. When a wheal and flare was observed, but did not satisfy the criteria, the reaction was recorded as trace and evaluated as negative.

Oral Prausnitz-Küstner tests (Walzer test)

Immediately after finishing the P-K test, a raw egg was given to the recipient orally and the P-T sites were observed for 4 hours. The evaluation of the result was as described. In some cases the test was repeated with a boiled egg.

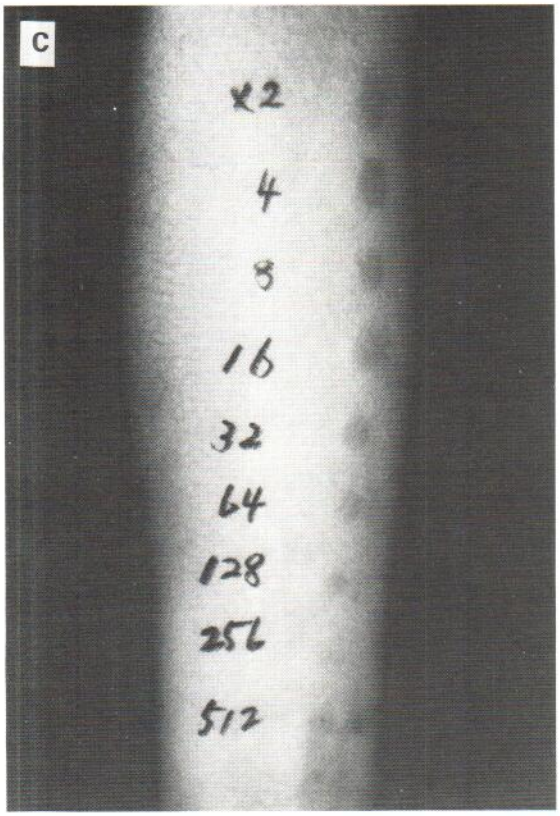
RESULTS

P-K reaction

The positive reaction usually started with blush and itching 1 to 7 min after antigen injection, reached maximum at about 15 min, and thereafter faded away slowly leaving a slight red edematous induration for several hours. Of the 50 patients, 19 showed no wheal and flare reactions at all or only trace reactions. Thirty-one showed positive reactions at one or more P-T sites depending on the intensity of the allergy. The titers of the positive reactions (P-K titer) were from 2 to 8192.

Oral P-K reaction

A typical positive reaction started first at the site of the lowest serum dilution with blush and itching, soon followed by a wheal and flare (Fig. 1 *a*) at about 1 hour (30 min to 2 h) depending on the intensity of the allergy. Then the positive reaction spread to the higher serum dilutions, reaching a maximum reaction



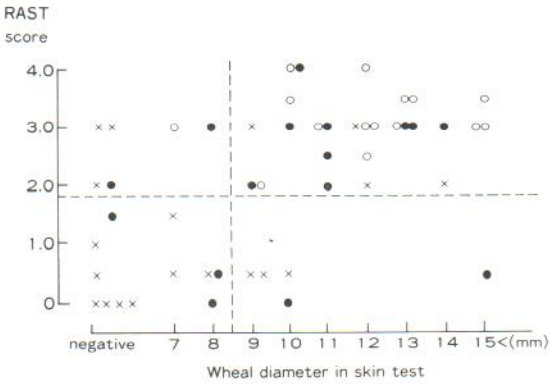


Fig. 2. The relation of the P-K test and the oral P-K test (Walzer test) to the wheal diameter in skin test and the RAST score. Open circle indicates positive both in the P-K test and the oral P-K test. Closed circle indicates positive in the P-K test but negative in the oral P-K test. Cross mark indicates negative in both tests.

at about 2 h (1–3 h), while the wheals and flares at the first positive tests were fading (Fig. 1 *b*). Thereafter, all wheals and flares waned slowly and left a slightly red edematous induration (Fig. 1 *c*) for several hours. Of the 50 cases studied, positive reactions were observed in 15 and their titers varied from 2 to 256.

The relation of the P-K test and the oral P-K test to the skin test and the RAST

The results of the P-K and oral P-K tests in relation to the results of the skin test and the RAST are summarized in Table I. In Fig. 2 the relation of the wheal diameter of the skin test and the RAST score is shown. Of 31 P-K positive cases, 23 were positive both in the skin test and the RAST, but the remaining 8 were negative in either or both of the skin test and the RAST. Of 15 oral P-K positive cases, 14 were positive both in the skin test of the RAST and 1 was skin test negative and RAST positive. There was no oral P-K positive case with negative RAST or with negative P-K test.

The relation of the P-K test and the oral P-K test

The P-K titers of 31 positive cases and the oral P-K titers of 15 positive cases as well as their relations are summarized in Fig. 3. All 5 cases with the low P-K titers of 2 or 4 were negative in the oral P-K test. Of 21 cases with the moderate P-K titers from 8 to 256, 12 were positive in the oral P-K reactions, and 3 cases with the high P-K titers of 1 024 or higher all showed positive oral P-K reactions. The ratio of the oral P-K titer and the P-K titer in each case was from 1 : 2 to 1 : 32.

Table I. *The relation of the P-K test and the oral P-K test to the results of the skin test and the RAST*

RAST skin test	(+) (+)	(+) (−)	(−) (+)	(−) (−)	Total
P-K test	(+) 23	3	2	3	31
	(−) 4	3	3	9	19
Oral P-K test	(+) 14	1	0	0	15
	(−) 13	5	5	12	35
Total	27	6	5	12	50

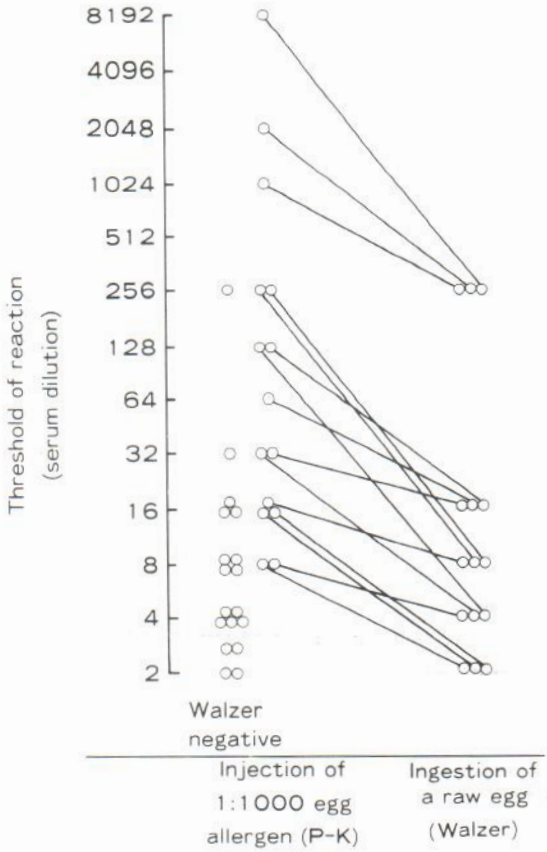


Fig. 3. Comparison of the titers (thresholds) of the positive reactions in the P-K test and the oral P-K test (Walzer test). The titers are shown by the maximum serum dilution with positive reaction. The two titers are lined in the cases positive in the both tests.

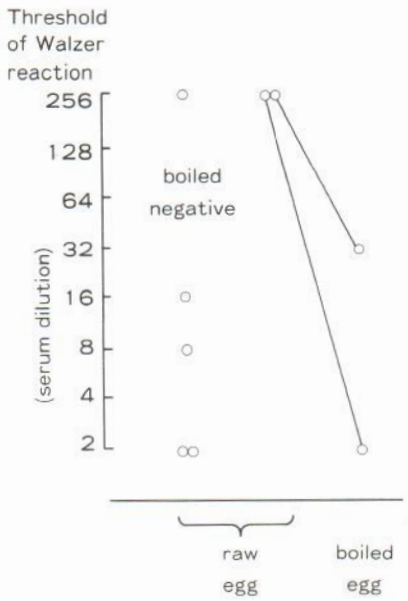


Fig. 4. Comparison of the oral P-K test (Walzer test) induced by a raw egg and by a boiled egg. The titers (thresholds) of the two cases positive in the both tests are lined.

Comparison of a raw egg and a boiled egg in the oral P-K test

In seven cases, the oral P-K test was repeated with an egg boiled for 10 min. A positive reaction was observed only in two. As shown in Fig. 4, the oral P-K titers of these 2 cases were 256 to a raw egg and 32 and 2 to a boiled egg, respectively. The time of appearance of the reaction after a boiled egg was similar to that with a raw egg. The remaining one case with the titer of 256 and the four cases with the titers between 16 and 2 to a raw egg were all negative to a boiled egg.

DISCUSSION

We demonstrate in this paper that serum from patients with atopic dermatitis passively transferred to the skin of healthy adults caused type I allergic reactions at the P-T sites in reaction to the antigens arriving there after the ingestion of a raw egg by the recipients. The strength of the oral P-K reaction was mostly between 1/32 to 1/2 of that of the skin P-K reaction. The total amount of egg antigens arriving at the skin during the oral P-K test is therefore estimated to be in the same range of antigen amount as is contained in 0.025 ml of the 1 : 1 000 egg allergen.

The percentage of the positive oral P-K reaction occurring with the serum of the patients who were shown to be allergic to hen's egg by the skin test or the RAST was surprisingly high (39.5%). This suggests that many egg allergic children with atopic dermatitis have IgE egg antibody levels in the serum sufficient to react with the ingested egg and cause type I allergic reactions. The patients studied in this paper were under the age of three years. In this age group raw eggs are rarely eaten, but they may get boiled or baked eggs. The antigenic activity of a boiled egg in comparison to a raw egg could be studied only in a few cases. From our data it may be suggested that the antigenic potency of a boiled egg is roughly 1/10 or 1/100 of a raw egg or less. If we assume that the ratio of the body weights of the children and the mothers is approximately 1/10, a boiled egg might be able to cause a typical type I allergic reaction in an egg allergic atopic child relatively often.

As compared to the ordinary P-K reaction which is caused by the antigen inoculated directly at the reaction site, the oral P-K reaction is induced by the antigen conveyed to the skin via blood vessels. This explains the time lag in the appearance of the urticarial reaction at different serum dilutions in the oral P-K test; the reaction first appeared at the site of the highest concentration and then at following concentrations. Walzer described the difference of the onset time of the oral P-K reaction by different foods (9). Reactions came earlier with herring and later with a raw egg. Our investigation confirm the late onset by a raw egg as well as a boiled egg.

Patients with atopic dermatitis have not only IgE antibody but also other classes of antibodies (IgG and IgA) to foods (8). What kind of functions these antibodies have are not known yet. But it is speculated that food antibodies of IgG or IgA classes might modify, perhaps inhibit, the symptoms delivered by IgE antibodies at various places of the body like in the digestive tract, the blood stream, in tissue fluid and on mast cells. In this study, the oral P-K test was performed two days after passive transfer of the patient's serum. Therefore, short-term skin sensitizing IgG antibodies (9) were thought to have disappeared from the P-T sites at the time, and the urticarial reaction we observed in the oral P-K tests were probably purely IgE-dependent.

The symptoms occurring in patients with atopic dermatitis after eating "allergic" foods are thought to be much more complicated than the one we have observed in the oral P-K test. Our investigation is only the first step to analyze the detailed mechanisms of food allergy occurring in atopic dermatitis.

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Studies of Atopic Patch Tests

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35 patients were patch-tested for 72 h with house dust mite, timothy- and birch-pollen, Cladosporium herbarum and hen's egg white allergens, prepared in a cream in concentrations up to 1 000 times the concentration used for skin prick testing. 6 patients developed a total of 10 positive reactions. All the strong positive patch-test reactions occurred in patients with a strong positive skin prick test to the same allergen. Immunohistochemical studies of biopsied positive patch-test reactions demonstrated a mononuclear cell-infiltrate in the upper part of dermis, consisting mainly of T-cells, with a slight predominance of T-helper-cells as compared to T-suppressor cells, and about 10 % CD1 positive cells. No significant responses were obtained in peripheral blood mononuclear cell-cultures stimulated with the various allergens. A positive patch-test reaction to birch-pollen was successfully transferred passively to a non-allergic-recipient, suggesting that the positive reaction may depend upon sensitizing factor(s) in the serum. ***Key words: Atopic dermatitis; Patch tests; Allergy; Passive transfer.***

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During the last few years, several papers have been published, dealing with positive epicutaneous patch-test reactions (EPR) to atopic allergens, such as house dust mite and birch-pollen, in atopic patients (1, 2, 3). The possibility that allergens, brought in direct contact with the skin, for example airborne, may contribute in the pathogenesis of the dermatitis, is an interesting aspect of the relationship between atopic dermatitis and allergic reactions.

In the present work we ask the question if atopic allergens elicit EPR. Furthermore, we ask the question if the allergen-specific EPR is associated with the presence of high levels of allergen-specific IgE, and if the various allergens are able to stimulate peripheral blood mononuclear cells (PBMC). Finally, we demonstrate passive transfer of EPR to a non-allergic recipient.

MATERIALS AND METHODS

Patients

35 patients with atopic eczema, aged 9–72, mean 26 years, were included in the study. The diagnosis was based upon criteria outlined by Hanifin & Rajka (4). The severity-degree of atopic dermatitis, assessed by means of clinical criteria described elsewhere (5), was found to be mild in 11 patients, moderate in 12 and severe in another 12 patients. All patients had +++ or stronger reactions in skin prick test (SPT) to at least two of the allergens used in patch-testing. One of the patients (R.S.) had repeatedly noticed aggravation of the dermatitis during the birch-pollen season. Apart from this, none of the patients had a history of aggravation of the dermatitis related to contact with the allergens used for patch-testing. However, most of the patients with positive reactions in SPT to timothy- and birch-pollen reacted with rhinitis when exposed to these allergens.

Antisera

OKT6 and OKT8 monoclonal antibodies were obtained from Ortho Pharmaceutical Corp., Raritan, N.Y. Anti-Leu 4, anti-Leu 3a + 3b, and anti-HLA-DR antibodies were obtained from Becton-Dickinson, Sunnivale, CA.

Serum for passive transfer of EPR

Serum was drawn from one of the atopic patients (R.S., 9 years old) with positive reactions in SPT as well as in EPR to timothy-pollen, birch-pollen and hen's egg white. Three 1.0 ml aliquots of this serum (R.S.-serum) were incubated with: 78 PRIST discs (anti-IgE), 63 g_6-RAST discs (timothy) and 72 T_3-RAST discs (birch), respectively, at room temperature (23°C), on a "Rock and Roller" overnight. The discs were removed from the sera (650–700 µl), and the sera were then sterile filtered using a Milex 0.2 µm filter. Untreated and absorbed R.S.-serum were tested for total IgE levels and specific IgE to timothy, birch and egg white, using Phadebas g_6, t_3 and f_1 discs, respectively.

Antigens

The hen's egg white antigen solution was prepared by diluting egg white from fresh eggs (less than 24 h old) with an equal volume of 0.15 mol/l NaCl (6). Freeze-dried allergens from timothy-pollen, house dust mite (D. farinae), cladosporium herbarum and birch pollen, were intermediate products for Spectralgen® (Pharmacia, Uppsala, Sweden), supplied by Nycomed (Oslo, Norway). The allergens were dissolved in 0.15 mol/l NaCl and then diluted in a fatty cream to the following concentrations (w/v): 0.3 g/l (house dust mite), 10 g/l (cladosporium herbarum), 2 g/l (birch-pollen), 1.6 g/l (timothy-pollen). These concentrations are up to 1 000 times the con-

centrations used in skin prick testing. Phazet® (Nycomed, Oslo, Norway) was used in SPT for all allergens, except for egg white, which was prepared as described.

Skin prick test (SPT)

SPT was performed using Phazet-histamine® as the positive reference (1 HEP), defined as +++; and Phazet-negative® as the negative reference. Reactions that were half the histamine-reference (defined as ++) or stronger, were recorded as positive (7).

Patch tests

Patch tests were performed using Finn Chambers® (Epitest Ltd., Helsinki, Finland), applying the various allergens at clinically normal skin on the back for 72 h. Two chambers served as controls, both containing the cream without any allergens added. In the passive transfer experiments the chambers were removed after 2, 6, 12, 72 and 120 h for recording of the reactions. Following the first four recordings, i.e. 72 h, the allergens were reapplied at the same test sites.

The patch-test reactions were recorded as +: erythema, ++: erythema and papules/oedema and +++: erythema, papules/oedema and vesicles.

Urticarial reactions observed in the passive transfer experiments were expressed as the products of the two widest perpendicular diameters of the wheals measured in mm.

Passive transfer test

The recipient was a non-allergic volunteer (one of the authors) being negative in SPT to timothy, birch and egg white and with total IgE in serum less than 10 U/ml. Each of the four R. S.-sera were used for intradermal infiltration of three different sites on the volar aspect of the forearm, each site being infiltrated with 0.1 ml serum. Infiltration with the untreated R.S.-serum in a skin area that was not patch-tested, served as a control. 24 h later, allergen preparations containing timothy-pollen, birch-pollen and egg white were applied so that each of the three allergens were tested against each of the four sera. As control, the allergens were also applied in skin areas that had not been infiltrated with R. S.-serum. All tests were read blindly.

Separation of PBMC

PBMC were separated by means of flotation on Lymphoprep® (Nycomed, Oslo, Norway) as described by Bøyum (8).

Cell culture techniques

Lymphoprep-isolated PBMC, in medium RPMI-1640 with L-glutamine (Gibco Bio-Cult, Glasgow, Scotland) supplemented with penicillin, streptomycin and 20% pooled human serum, from 8 patients with atopic dermatitis and positive SPT as well as patch tests to some of the various allergens, and 3 controls, were incubated with various dilutions (range 1 mg/ml – 1^{-10} mg/ml) of timothy-pollen, birch-pollen, house dust mite (D. farinae), cladosporium herbarum and hen's egg white allergens in round-bottomed microtitre plates ($5 \cdot 10^4$ and 10^5 cells per well) for 6 days in a humid 5% CO_2 atmosphere. ^{3}H-thymidine was added 18 h before harvesting with a semi-automatic multiple cell culture harvester (Skatron, Lierbyen, Norway). Incorporation was measured by means of a liquid scintillation counter and expressed as mean ± SD of triplicates. PBMC cultures without antigen served as negative controls, and cultured with PPD as positive controls. All the patients were tuberculin sensitized through vaccination. PBMC from tuberculin sensitive individuals cultures with purified protein derivative of tuberculin (PPD) served as additional positive controls.

Immunohistochemical studies

Biopsy specimens were taken from a positive EPR to timothy-pollen in one of the patients (S. R.) and the passively transferred EPR to birch-pollen. The samples were embedded in OCT (Tissue Tek), snap-frozen in liquid nitrogen and stored at −20°C.

Cryostat sections of the skin biopsy samples were incubated with the various monoclonal antibodies in the alkaline phosphatase anti-alkaline phosphatase (APAAP) staining technique as described by Cordell et al. (9). The numbers of positively stained cells were estimated in a light microscope.

Radio-allergosorbent test (RAST) and Paper immunosorbent test (PRIST)

Specific and total IgE in serum were determined by means of RAST and PRIST, respectively, according to the recommendations of the manufacturer (Pharmacia, Uppsala, Sweden).

RESULTS

EPR

Positive EPR were recorded in 6 patients (17%) (Table I). Except for a weak EPR to house dust mite in one patient, all other reactions were accompanied by positive reaction to the same allergen in SPT.

Total and allergen-specific IgE in R. S.-serum

The results from determination of total and allergen-specific IgE in the 4 different R. S.-sera, are presented in Table II. The results show that the adsorption of IgE using PRIST discs, significantly reduced both total- and specific-IgE levels. Adsorption of specific IgE using RAST discs did, however, only reduce the concentration of IgE specific for the allergen on the absorbing discs.

Stimulation of PMBC with the various allergens

No stimulation was obtained with the PBMC from the patients with a range of concentrations of the various allergens, while stimulation was obtained after stimulation with PPD.

Immunohistochemical studies of positive EPR

The immunohistochemical studies of the EPR demonstrated a dermal infiltrate consisting of approximately 90% T-cells, with a slight predominance of CD4 positive cells as compared to CD8 positive cells. Approximately 90% of the cells in the dermal infiltrate were HLA-DR positive, and about 10% carried

CD1 antigens. There were also increased numbers of CD1 and HLA-DR positive cells in the epidermis.

Passive transfer experiment

2–12 h after the application of the allergens in the passive transfer experiment, a number of urticarial reactions appeared at the various test sites, as shown in Table III. At 72 h a positive EPR against birch-pollen had developed at the test site that had been infiltrated with the R. S.-serum containing the lowest amount of antibodies against birch. This test site was the only one being tested with birch, that did not develop an urticarial reaction during the first day (Table III). The positive EPR, appearing as itchy erythema with small papules, increased until day 5, when a biopsy specimen was taken. Light microscopical investigation demonstrated a mononuclear, perivascular infiltrate in the upper part of dermis and

Table I. *Patients with positive epicutaneous patch reactions*

ERP = epicutaneous patch reaction. SPT = skin prick test

Patient/age	Allergen	EPR	SPT
R. S./9[a]	Birch	+++	>++++
	Timothy	+++	>++++
	Egg white	+++	+++
S. R./33[a]	Timothy	+++	++++
O. R./25	Birch	++	+++
E. K./20	Cladospore	+	+++
	House dust mite	+	–
P. B./10[a]	Birch	+++	+++
H. G./18	Birch	++	+++
	Egg white	++	++

[a] These patients were retested. All reactions were reproduced.

Table II. *Concentrations of total IgE and specific IgE to timothy, birch and egg white in untreated R. S.-serum and R. S. serum pre-incubated with PRIST discs and g_6-, t_3- and f_1-RAST discs*

		R. S.-serum incubated with		
Test	Untreated R. S.-serum	PRIST IgE discs	RAST g_6 timothy discs	RAST t_3 birch discs
PRIST IgE (total)	1 668 U/ml	164 U/ml	1 376 U/ml	1 772 U/ml
RAST g_6 timothy	23.1 PRU/ml	7.7 PRU/ml	3.5 PRU/ml	28.7 PRU/ml
RAST t_3 birch	21.7 PRU/ml	3.5 PRU/ml	27.3 PRU/ml	14.7 PRU/ml
RAST f_1 egg white	13.3 PRU/ml	1.3 PRU/ml	13.3 PRU/ml	13.3 PRU/ml

Table III. *Urticarial lesions[a] during 2–12 h in passive transfer experiment*

	R. S.-serum used from infiltration of test site				
Allergen	Untreated	Low tot-IgE	Low anti-birch	Low anti-timothy	No serum (controls)
Egg white	25	0	100	64	0
Birch	100	100	4	60	0
Timothy	16	4	0	0	0

[a] Urticarial lesions are expressed as the products (mm^2) of the two widest perpendicular diameters of the wheal.

slight spongiosis in epidermis. Immunohistochemical studies of the dermal infiltrated showed a pattern essentially similar to that observed in the positive EPR in the patient.

DISCUSSION

We succeeded in provoking positive EPR to all the allergens used in patch-testing. There is no simple method to decide whether the positive EPR were allergic reactions or simply due to irritation by the test preparations. The following observations indicate that the positive reactions depended upon allergy and not irritation: Firstly, there were only a few positive reactions, 10 out of 175 tests. Secondly, it was not the same allergen preparation that elicited all or most of the reactions. Except for a weak ERP to house dust mite allergens, all the positive reactions appeared in patients being sensitized to the allergen, presenting positive reaction to the allergen in SPT. Thirdly, a positive EPR to birch was transferred passively to a non-allergic recipient.

The immunological basis for the positive EPR is not clear. The presence of specific IgE against the allergen in patients with positive EPR, which is in accordance with other reports (1, 3), may suggest that IgE-antibodies are involved. Since, however, a number of patients with strong IgE-mediated reactions to the allergens did not develop positive EPR, the possibility exists that the positive reaction might not be dependant on allergen-specific IgE to occur, but mainly on another or additional factor. The successful passive transfer of a positive EPR might suggest that an additional factor is present in serum, and that this factor is capable of sensitizing the skin of the recipient.

The finding that the only test site reacting with a positive EPR to birch allergens was the one that had been injected with the serum containing least antibodies against birch-pollen, supports such a hypothesis.

The various allergens did not stimulate lymphocyte proliferation of the PBMC cultures obtained from the patients with positive EPR to the same allergens. Antigen stimulation of T-cells requires that the antigen is presented by an accessory antigen-presenting cell. PBMC are easily in-vitro stimulated by antigens like PPD (11) and in most cases also with other antigens, for example herpes simplex virus (12). Stimulation of T-cells with nickel sulphate in nickel sensitive subjects however, is often weak or negative when peripheral blood monocytes/macrophages are used as the antigen-presenting cells. Using epidermal Langerhans cells as antigen-presenting cells, in comparison, induce strong nickel-specific T-cell responses in the same subjects (13), indicating that Langerhans cells may be highly specialized in presenting certain antigens to T-cells. Since positive EPR may involve and need antigen presentation by Langerhans cells to occur, the lack of Langerhans cells in the PBMC-cultures may explain the negative reactions, similar to the findings with nickel sulphate. If an unknown serum factor present in the patients sera is necessary for the reaction to occur, another explanation might be that we used 20% pooled human serum (not from the patients) in the culture medium. Further studies are under way to elucidate this.

In allergic as well as irritant contact dermatitis the predominant cell type present is the T lymphocyte with variations in the helper/suppressor T-cell ratios between patients and within time periods (10). In the positive EPR to timothy we found a predominance of T-lymphocytes, but a considerable number of CD1 positive cells, in all probability Langerhans cells, were present in the dermal infiltrates, and increased in epidermis. The Langerhans cells are antigen presenting cells for T-cells (11), and the presence and increased number of Langerhans cells may reflect an antigen presenting function in the positive timothy reaction. Our results are in agreement with those of Reitamo et al. (3) who biopsied positive reactions to birch-pollen and house dust mite.

The allergens, being applied directly on clinically unaffected skin, are apparently capable of penetrating the epidermis, although most of them are molecules greater than 10000 daltons. This is also indicated by the development of urticarial reactions in the passive transfer experiment. The observation that some patients also react to these allergens with positive EPR suggests that such allergens may be of significance in the pathogenesis of atopic dermatitis in some patients.

ACKNOWLEDGEMENT

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In Vitro IgE-secretion in Atopic Eczema: Influence of Allergens and Mitogens and Role of CD8 T Cell Subpopulation

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In cultures of peripheral blood mononuclear cells (PBMC) from 23 atopic patients and 14 controls the influence of mitogens, allergens and CD8 suppressor T lymphocytes on the in vitro IgE response was studied. The in vitro IgE levels in lymphocyte culture supernatants reached a plateau after 6 days of culture, whereby low levels of IgE could be reproducibly measured down to 0.5 ng/ml. The spontaneous in vitro IgE secretion from PBMC of atopic eczema patients was elevated in comparison to the control group and showed a direct correlation ($r=0.72$) to the serum IgE levels. Pokeweed mitogen rather suppressed the in vitro IgE production. After removal of the CD8 subpopulation of T lymphocytes by using an indirect erythrocytes rosetting technique we found increased in vitro levels pointing to a role of IgE regulating T suppressor subpopulations.

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Although much has been learned about the regulation of IgE synthesis in rodent species (1, 5), little is known about the mechanisms involved in the control of human IgE production, since analogous in vivo studies are not possible.

Lymphocyte culture studies in atopic patients (especially patients with atopic eczema) showed elevated spontaneous in vitro IgE levels (2, 4, 11) with controversial results on the influence of antigenic or mitogenic stimulation (9, 11, 14, 18). Disturbed T cell mechanism has been described to be of importance for the high IgE synthesis in atopic people. Patients with atopic eczema have been reported to possess low T cell levels (8) as well as depressed T cell functions (16) or reduced T suppressor cell numbers (7).

Here we studied the influence of allergens and mitogens upon the in vitro IgE secretion and the effect of depletion of a T cell subpopulation.

MATERIAL AND METHODS

Cell suspensions

Heparinized venous blood was obtained from 23 atopic patients (among them 15 patients with atopic eczema only) and 14 non-allergic volunteers. Peripheral blood mononuclear cells (PBMC) were subsequently isolated by Ficoll-Paque density gradient centrifugation (Pharmacia, Uppsala, Sweden). CD8 T cells were removed by first incubating the PBMC with the mouse monoclonal antibody MT-811 (anti CD8, IgGl isotype), subsequent rosetting with goat anti mouse Ig bearing ox erythrocytes (10) and final Percoll density gradient centrifugation (Pharmacia, Uppsala, Sweden).

Culture conditions

Cells (1×10^6/ml) were suspended in RPMI 1640 growth medium supplemented with 10% fetal calf serum, 1% penicillin/streptomycin and 1% L-glutamine (Seromed, Munich and Gibco Europe, Karlsruhe, FRG) and cultured up to 6 days at 0.2 ml per well in round-bottomed microtiter plates (Bioplast, Berlin, FRG). Stimulation was performed by adding the following lectins and antigens in a 10 µl volume of culture medium: Purified phytohemagglutinin (PHA), 1 µg/ml (Gibco Europe, Karlsruhe, FRG), concanavalin A (ConA), 3 µg/ml (Roth, Karlsruhe, FRG), pokeweed mitogen (PWM), 1 µg/ml (Seromed, Munich, FRG), cat epithelium 0.5% w/v 1 : 10 (Beecham-Wülfing, Neuss, RFT). Control cultures contained medium only. At the end of the culture period IgE concentration in respectively 0.1 ml of cell-free supernatant was determined by a modified PRIST technique (12) (Pharmacia, Uppsala, Sweden).

RESULTS

Correlation between in vitro and serum IgE

Freshly isolated PBMC from 15 patients with atopic eczema were kept in culture without stimulation. The IgE secreted after 6 days was measured in the supernatants and compared with the serum IgE levels. The results are shown in Fig. 1. All supernatant fluids showed measurable IgE levels. Spontaneous IgE se-

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Parts of these data have been presented at the Joint Meeting of the E.S.D.R. and S.I.D., Geneva, 1986.

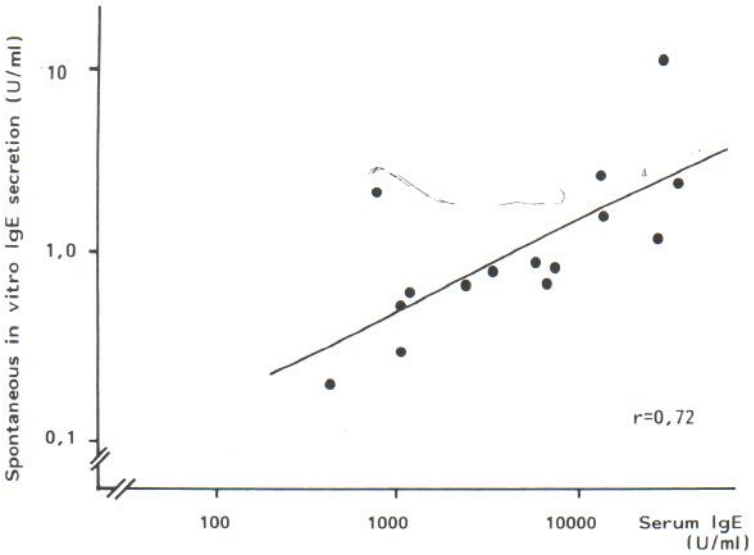

Fig. 1. Correlation of serum and in vitro IgE concentration in patients with atopic eczema ($n=15$).

cretion and corresponding serum IgE level were significantly correlated ($r=0.72$, $p<0.01$).

Kinetics of in vitro IgE secretion

PBMC from 4 atopic eczema patients were stimulated with PHA, PWM and grass pollen extract. After 2, 4 and 6 days the IgE levels in the supernatants were measured. Most of the in vitro IgE levels came to a plateau after 4 days. Stimulation with PWM resulted in inhibition of in vitro IgE production, allergen led to an increase of the in vitro IgE secretion after prolongated culture in single patients (Fig. 2). Patients with marked in vitro IgE response after antigenic stimulation generally showed a specific sensitization

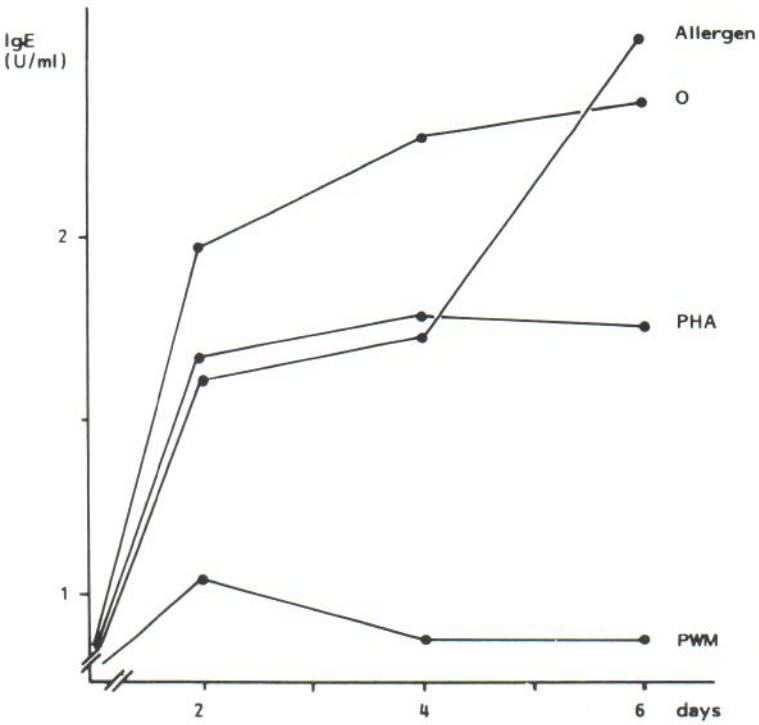

Fig. 2. Kinetics of in vitro IgE secretion ($n=4$).

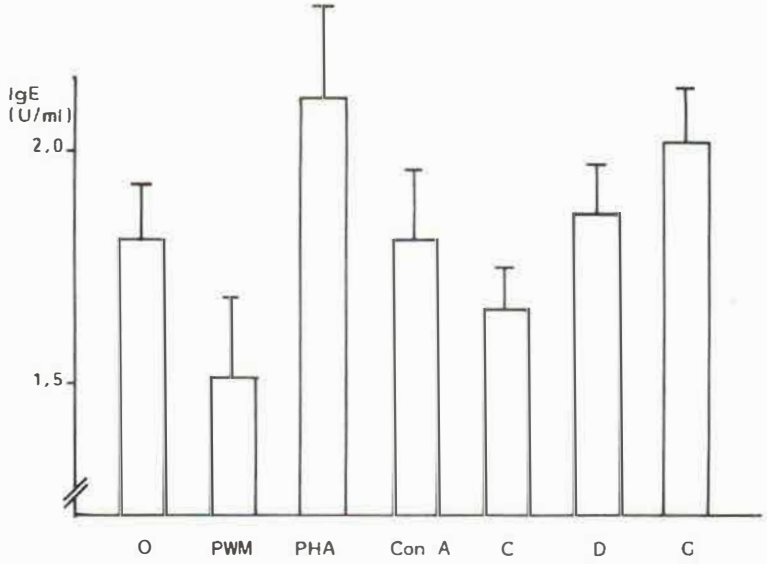

Fig. 3. Influence of mitogens/allergens upon in vitro IgE secretion of patients with atopic eczema ($n=15$). Because of minimal IgE levels controls are not shown. C = cat epithelium, D = house dust mite (D. pteronyssinus), G = grass pollen.

towards the same allergen in the radio-allergo-sorbent-test (RAST) (Table I).

Effect of mitogen or allergen stimulation on in vitro IgE secretion

To determine the effect of different lectins and allergens, we conducted experiments in which IgE secretion from unfractionated mononuclear cells after stimulation was compared with the spontaneous in vitro IgE secretion. The results of these studies are presented in Fig. 3. Cells from normal controls showed very low in vitro IgE levels with a modest augmentation after addition of PWM, while other lectins and antigenic stimulation had no remarkable effect. The spontaneous in vitro IgE secretion of atopic dermatitis patients was inhibited by PWM and ConA allergen eczema was of no statistically significant effect.

Influence of CD8 cell depletion

To examine the influence of CD8 T lymphocytes on the in vitro IgE secretion, unfractionated and CD8-cell-depleted (less than 1%) PBMC from 7 healthy donors and 19 atopic patients were simultaneously kept under identical culture conditions. T and B cell ratios were kept equal. The IgE production after 6 days of culture from fractionated and unfractionated cells of the same patients was measured and compared. PBMC of atopic patients demonstrated higher spontaneous in vitro IgE secretion after T suppressor cell depletion (Fig. 4). Likewise, CD8 depletion resulted in augmentation of lectin and allergen-stimulated in vitro IgE (Table II). Some of the controls now

Table I. *Six patients with atopic eczema in whom spontaneous in vitro IgE secretion was clearly enhanced by allergen stimulation*

Allergen	Serum RAST (class)	Increase in in vitro IgE (U/ml)
D. pteronyssinus	2	0.4
	3	1.58
Grass pollen	4	4.00
	3	0.50
	0	0.49
		0.40

Table II. *Influence of CD8 depletion upon in vitro IgE secretion from PBMC of atopic patients ($n=19$)*

	Spontaneous	PWM	PHA	Allergen (cat)
Increase	11	10	10	11
Decrease	5	4	3	6
Unchanged	3	5	6	2
	$p<0.05$	$p<0.05$	NS	NS

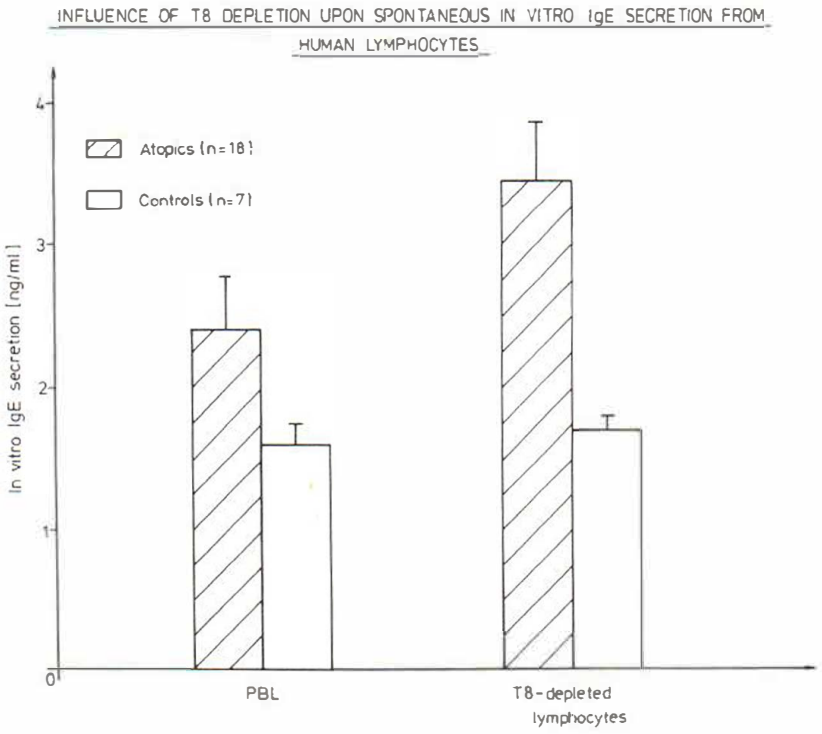

Fig. 4. Spontaneous in vitro IgE secretion in atopics ($n=7$) and normals ($n=10$) after depletion of CD8-T cells by indi-

secreted detectable amounts of spontaneous in vitro IgE (data not shown).

DISCUSSION

Tada et al. (17) first pointed to the role of T cell regulatory mechanisms on IgE biosynthesis when they demonstrated increase of ongoing IgE antibody production in rats after treating the animals with antithymocyte serum, whole body irradiation, adult thymectomy or with various immunosuppressive drugs.

When lymphocyte cultures are used to study human IgE regulation (13), 6 or 7 days of culture are required as our data showed. A recent multicenter study in which our laboratory took part reported a low reproducibility of in vitro IgE measurements below 0.5 ng/ml and stressed the need for appropriate culture conditions and IgE detection methods (3).

Our observation that atopic eczema patients with high serum IgE concentrations also showed a high spontaneous in vitro IgE secretion suggests that the trigger to increased B cell IgE synthesis in vitro may already have occurred in vivo (15). Thus the addition of lectins—especially PWM—could not further increase but rather inhibit spontaneous in vitro IgE production (11, 14).

The marked augmentation of in vitro IgE secretion after removal of CD8 suppressor T cells points to the existence of IgE-isotype-specific regulatory T lymphocytes within this subpopulation. A functionally altered suppressor cell population, extensively investigated by Ishizaka et al. (6) in a rodent model, may be one factor in the impaired T–B-cell cooperation leading to atopic disease. The model of spontaneous in vitro IgE secretion in lymphocyte cultures might be suitable to study possible inhibitory factors of human IgE production.

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Heterogeneous Distribution of Mast Cells in Lichenifield Lesions of Atopic Dermatitis

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The aim of the present study was to determine whether the number of mast cells in lichenified lesions of atopic dermatitis (AD) have a relationship to familial background of atopic respiratory disease (ARD). We obtained biopsy specimens of lichenified lesions from 59 consecutive patients with AD. They included 35 AD patients who had a personal history of ARD, 9 AD patients without a personal history of ARD but with a family history of ARD, and 15 "pure" AD patients without a personal or a family history of ARD. From each biopsy specimen, 4 μm-thick paraffin-embedded sections and/or 1 μm-thick Epon-embedded sections were prepared, and stained with Giemsa's reagent. With both methods of preparation, the sections from AD patients with a personal history of ARD showed significantly greater mast cell number in lichenified lesions than the "pure" AD patients. It is suggested that the increase of mast cells often seen in AD lesions may be characteristic of a subgroup of AD patients who have a predisposition for ARD.

Several authors (1–3) have reported that mast cell numbers are increased in lichenified lesions of atopic dermatitis (AD). But others (4, 5) state that skin lesions of AD often show a normal number of mast cells. Thus, at present it is unclear whether the increase of mast cells in skin lesion is a common feature of AD or whether the increase of tissue mast cells occurs only in a subgroup of AD patients.

Mast cells are known to play a part in the asthmatic reaction (6, 7). A recent report (8) showed that the number of mast cells or basophil progenitors in the circulation are increased in atopic patients. In the present study, therefore, we investigate whether the presence of a personal or family history of atopic respiratory disease (ARD) is associated with an increase of mast cells in lichenified lesions of AD.

MATERIALS AND METHODS

Patients. A total of 59 patients with AD not being treated with topical corticosteroids for at least one month prior to the examination, were included in the study. The diagnosis of AD was based on the morphology and distribution of skin lesions, the chronic course, and a family history of AD or atopic respiratory disease (ARD). All patients had lichenified lesions in the antecubital and popliteal fossae, neck, and other predisposed areas. They ranged in age from 13 to 64 years, with a mean age of 23. They were classified into three subgroups: 1) those who had a personal history of ARD (35 cases), 2) "pure" AD patients without personal or family history of ARD (15 cases), and 3) those without a personal history of ARD but with a family history of ARD (9 cases).

Biopsies. In each of the 59 patients, one or two biopsy specimens were taken from a lichenified plaque.

A) *4 μm-thick paraffin-embedded sections.* Forty-nine biopsy specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. From each specimen 20 to 40 serial sections (4 μm) were prepared and stained with Giemsa's reagent.

B) *1 μm-thick Epon-embedded sections.* Thirty-two biopsy specimens were fixed with 3% glutaraldehyde in 0.1 M PBS at pH 7.4. Post-fixation in 1% Osmium tetroxide was followed by dehydration and embedding in Epon. Following the methods of Mihm et al. (2), 20 to 40 serial 1 μm-thick sections were prepared, and stained with Giemsa's reagent.

Counting of mast cells. All biopsy specimens had 1) perivascular infiltrates of mononuclear cells at interfollicular areas of upper dermis where the infiltrates were distributed in a horizontal direction, and 2) vertical distributed inflammatory cells around hair follicles. We counted the mast cells in a total of 1 000 cells at the interfollicular areas alone.

RESULTS

A) *4 μm-thick paraffin-embedded sections.* 1) In AD patients with a personal ARD history (31 cases) many mast cells were present in the lichenified lesions (Fig. 1). The mean number of mast cells was 58.5. 2) In "pure" AD patients without a personal or family history of ARD (12 cases), the number of mast cells were not increased (Fig. 2). The mean number of mast cells was 27.5. 3) In AD patients without a personal history of ARD but with a family history of ARD (6 cases), the number of mast cells in lichenified lesions varied widely from patient to patient. The mean number of mast cells was 54.9.

B) *1 μm-thick Epon-embedded sections.* 1) In AD

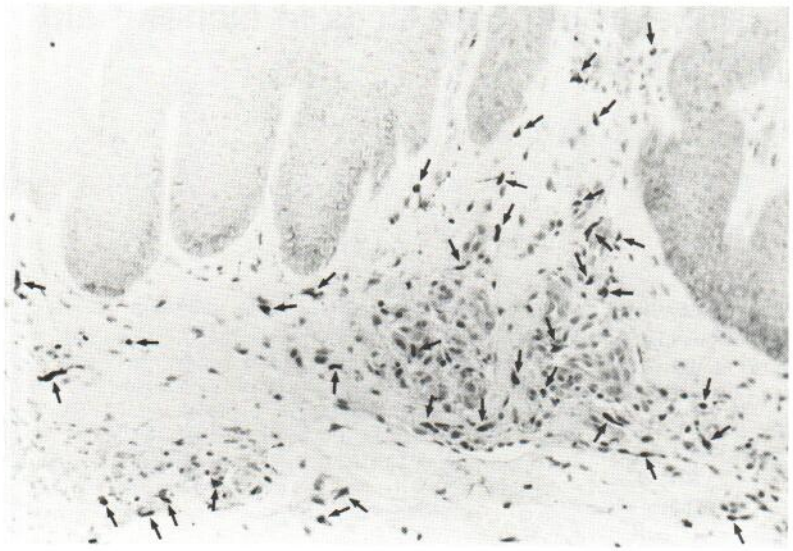

Fig. 1. 4 μm-thick section. Giemsa's staining. Lichenified lesions of AD patient with a personal history of ARD. Many mast cells are seen in the dermal infiltrate.

patients with a personal history of ARD (17 cases), mast cells were abundant in the lichenified lesions (Fig. 3). The mean number of mast cells was 60.2. 2) In "pure" AD patients (9 cases), the number of mast cells was not increased (Fig. 4). The mean number of mast cells was 24.8. 3) In AD patients without a personal history of ARD but with a family history of ARD (6 cases), the distribution of mast cells in the dermal infiltrate was similar to that in the "pure" AD group. The mean number of mast cells was 31.2.

Fig. 5 shows the distribution of mast cells in lichenified lesions in all the AD patients examined in the present study.

Thus, from the data of both paraffin-embedded sections and Epon-embedded sections, it was evident that there was a significant difference ($p<0.01$) in mast cell number in lichenified lesions between AD patients with a personal history of ARD and "pure" AD patients without a personal or a family history of ARD.

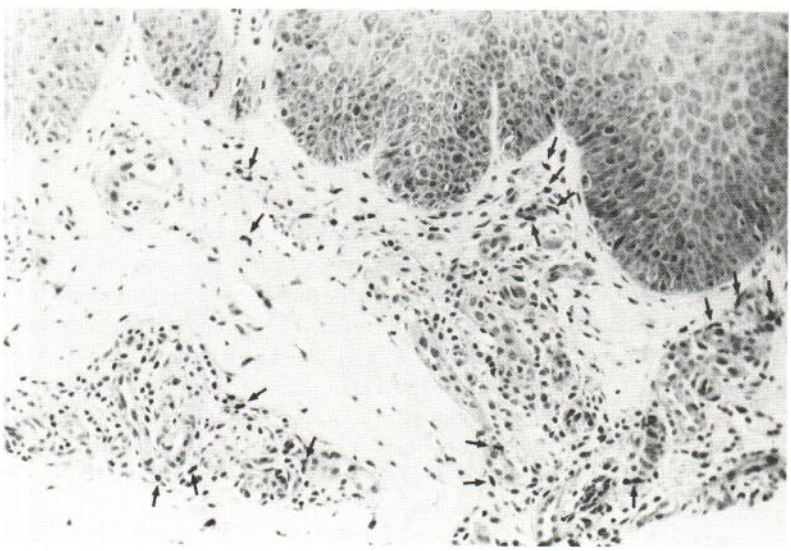

Fig. 2. 4 μm-thick section. Giemsa's staining. Lichenified lesion in "pure" AD patient. Only a small number of mast cells are present in the dermal infiltrate.

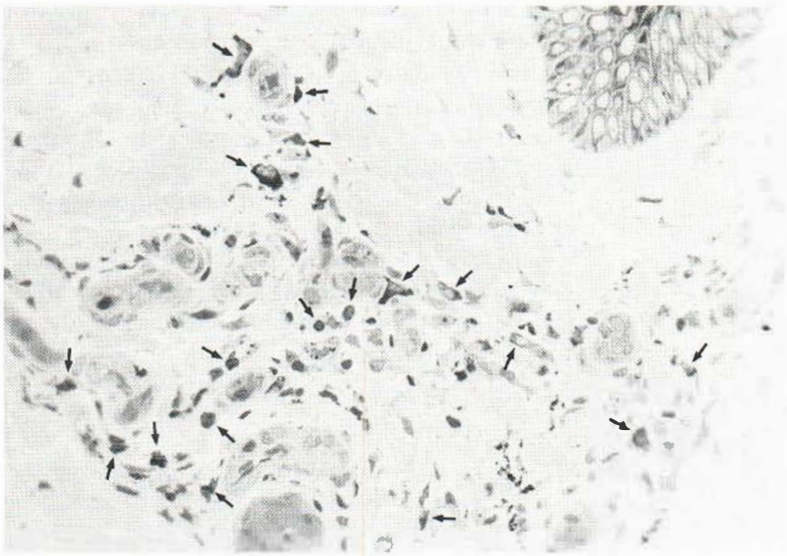

Fig. 3. 1 μm-thick section. Giemsa's staining. Lichenified lesion in AD patient with a personal history of ARD, showing many mast cells in the infiltrate.

DISCUSSION

The present study demonstrates that the number of mast cells in lichenified lesions of AD vary widely from patient to patient. The mast cell number was significantly higher in the group of AD patients with a personal history of ARD than in the "pure" AD patients without a personal or family history of ARD. Thus, it is likely that co-existent ARD is an important factor which leads to increased mast cell numbers in skin lesions of AD. A very high number of mast cells in lichenified lesions was observed in some patients of the AD group with only a family history of ARD. This may imply that the mast cell number in skin lesions are increased in AD patients who have a subclinical ARD, or predisposition to ARD.

Braun-Falco et al. (1) reported that an increase in mast cells was seen in lichenified lesions of AD. Mihm et al. (2), using 1 μm-thick Epon-embedded sections, also investigated the distribution of tissue mast cells in skin lesions of AD, and they concluded

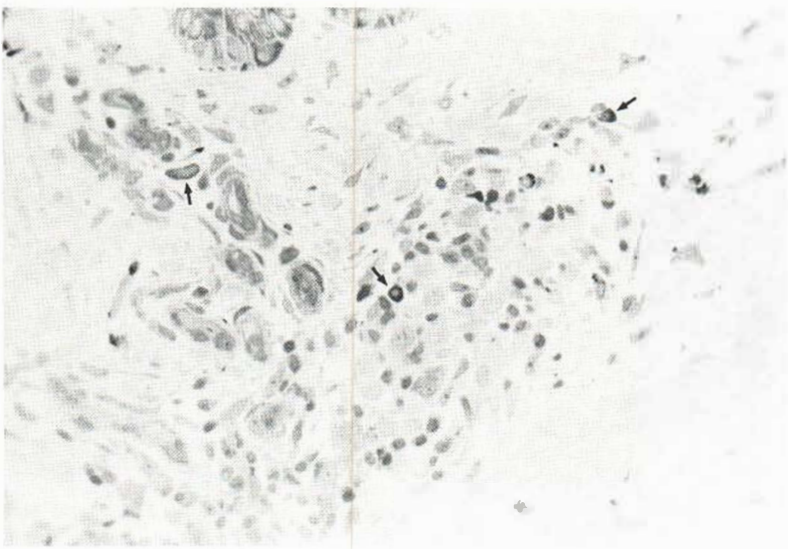

Fig. 4. 1 μm-thick section. Giemsa's staining. Lichenified lesion in "pure" AD patient. Only three mast cells are observed in the field.

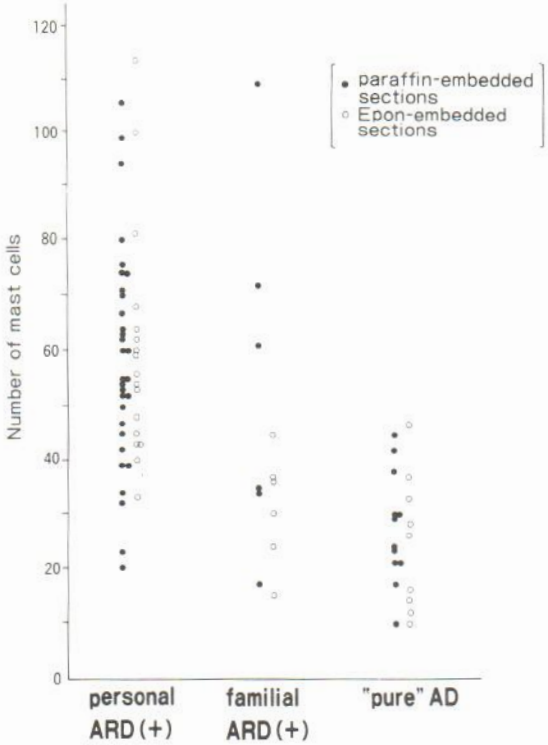

Fig. 5. Distribution of mast cells in lichenified lesions in the three subgroups of the AD patients.

that the number of mast cells in lichenified lesions of AD was strikingly increased. But these reports did not comment whether their patients had a personal or a family history of ARD.

On the other hand, Montgomery (4) concluded that the increase of mast cells was not always seen in lichenified lesions of AD patients. Braathen et al. (5) found no pathologically increased numbers of mast cells in the lesions of AD. Unfortunately, both these studies lacked an accurate description of familial background with regard to ARD in their patients.

In summary, from the results of the present study and previous reports (1, 2, 4, 5), we may conclude that the increase of mast cells often seen in lichenified lesions of AD is a feature of a subgroup of AD patients who have a predisposition for ARD.

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Patch Test Reactions to Inhalant Allergens in Atopic Dermatitis

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To study whether inhalant allergens could induce eczematous reactions on normal skin of atopic patients we applied birch pollen and house dust mite antigens at 500 times the concentration used for prick testing as epicutaneous tests. Six out of 17 patients with atopic dermatitis in remission had positive delayed type reactions to birch pollen and three to house dust mite. Only one out of 13 atopic patients without history of atopic dermatitis but with seasonal allergic rhinitis had a positive patch test reaction to birch pollen and no patient had positive test reactions to house dust mite. No positive patch test reactions to birch pollen or house dust mite were seen in the ten healthy control subjects. In patients with positive test reactions biopsies from the test sites revealed epidermal spongiosis and vesiculation. Immunostaining of the epidermis revealed keratinocytes displaying both CD1 and HLA-DR. The present study suggests that inhalant allergens can exacerbate atopic dermatitis. *Key words: Inhalant allergens; Birch pollen; House dust mite; Patch test reactions.*

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Many patients with atopic dermatitis show skin reactions of the immediate type, i.e. positive prick test reactions, when tested with various inhalant allergens. There is generally a good correlation between positive prick test reactions and immediate atopic symptoms such as allergic rhinitis. However, it has proved difficult to correlate these positive prick test reactions to the patient's eczema, which is a delayed type reaction. So far delayed type skin reactions have been mainly ascribed to house dust mite (1, 2).

We recently described a group of patients with a birch pollen allergy of the immediate type but who also showed seasonal exacerbation of the atopic dermatitis during the spring birch pollen season (3). The present study investigates patch test reactions to birch pollen and house dust mite in these patients and in other patients with atopic dermatitis. In addition to findings reported earlier (3) some new results on HLA-DR and CD1 expression of keratinocytes will be presented.

PATIENTS AND METHODS

Patients and control subjects

We studied 17 patients with atopic dermatitis. Thirteen of these patients had positive prick test reactions to birch pollen, and nine had positive prick test reactions to house dust mite. As controls we studied 13 atopic patients without dermatitis, 11 of whom had positive prick test reactions to birch pollen. As further controls we studied nine healthy subjects.

Allergens

We used lyophilized preparations of Aquagen SQ 108 birch pollen (*Betula verrucosa*) and Aquagen SQ 503 house dust mite (*Dermatophagoides pteronyssinus*). The allergens were purchased from Allergologisk Laboratorium A/S, Copenhagen, Denmark. As vehicles we used distilled water and white petrolatum. The allergens were used at 500-fold concentration as compared to the standardized prick test to 1 HEP (histamine equivalent prick). White petrolatum was used as control.

Patch testing and skin biopsies

The patch tests were performed with Finn Chambers (Epitest Ltd., Helsinki, Finland) applied to clinically normal skin on the back. The chambers were removed after 48 h. The patch test reactions were read at 48 h and optionally after 72 and 96 h.

Skin biopsies were taken from both positive epicutaneous test reactions and control sites.

Processing of skin biopsies

The biopsies were divided into two parts. One was processed for immunohistochemistry for quantitation of inflammatory cell subtypes; the other was used for quantitation of mast cells and basophils according to the methods described by Dvorak et al. (4) and Hénocq and Gaillard (5).

Immunostaining

The following primary antibodies were used: OKT3, OKT4, OKT6, OKT8, OKT9, OKM1, OKIa1 (all from Ortho Diagnostic Systems Inc., Raritan, NJ, USA), Leu7 (Becton-Dickinson, CA, USA) and anti B cell antibody (Dakopatts A/S, Copenhagen, Denmark). The immunoperoxidase staining was performed with the avidin-biotin-complex (ABC) method (Vectastain ABC kit mouse IgG PK-4002, Vector Laboratories Inc.; Burlingame, CA, USA) as described by Hsu, Raine and Fanger (6). The sections were incubated in the

Table I. *Positive test results from skin testing with birch pollen*

Clinical diagnosis	Number of patients	Positive prick tests	Positive patch tests
Atopic dermatitis	17	13	6
Allergic rhinitis (no eczema)	13	11	1
Healthy	10	0	0

dark with 0.5% 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., MO, USA) and 0.01% H_2O_2 in phosphate-buffered saline (PBS), counterstained with haematoxylin, dehydrated in a graded alcohol series, cleared in xylene and mounted in Histoclad (Clay Adams, NJ, USA).

The immunostaining was controlled as follows: 1) the first antibody was replaced with PBS or a nonrelevant monoclonal antibody and 2) tissue sections were stained for endogenous peroxidase activity without antibody treatment.

Mast cells and basophilic granulocytes

Mast cells and basophils were stained in paraffin sections with toluidine blue and Giemsa's stain.

RESULTS

Patch test reactions

Positive patch test reactions to birch pollen were seen in six out of 17 patients with atopic dermatitis and in one out of 13 patients with allergic rhinitis without dermatitis (Table I). All six patients with positive patch test reactions had positive prick test reactions to birch pollen. The only patient with a positive patch test reaction to birch pollen without atopic dermatitis had a positive prick test reaction to birch pollen. No positive patch test reactions to birch pollen or house dust mite were seen in the healthy subjects. Three out of nine patients with positive prick test reactions to house dust mite had positive epicutaneous test reactions to the mite. All three patients had positive epicutaneous test reactions to birch pollen as well.

Histopathology

In all patients with positive patch test reactions the biopsy from the test site revealed an eczematous skin reaction with epidermal spongiosis and vesiculation.

Keratinocyte staining for HLA-DR and CD1

We saw positive immunostaining of keratinocytes for HLA-DR and CD1 in all five patients with positive patch test reactions (Table II).

Immunostaining of the dermal infiltrate

Immunostaining of frozen sections with monoclonal antibodies showed dermal cell infiltrates consisting mainly of T cells with a CD4 to CD8 ratio of 2–6/1. 50–90% of the infiltrating cells were HLA-DR positive. There was a smaller proportion (0–30%) of CD1-positive Langerhans cells or indeterminate cells in the dermal infiltrate.

Mast cells and basophilic granulocytes

The proportion of mast cells and basophils in tissue sections was usually 5–10% and never exceeded 15%.

DISCUSSION

Platts-Mills and co-workers (1, 2, 7) have earlier shown that by using the house dust mite antigen P1 it is possible to produce a delayed type of response both in vivo as a patch test reaction and in vitro as blast transformation in atopic patients with positive prick test reactions to the same antigen. It has also been shown in a double-blind, controlled study that repeated exposure to house dust mite antigen produces a mild eczematous reaction of both eczematous and clinically uninvolved skin (8). Other workers have also confirmed that house dust mite or other inhalant allergens exacerbate atopic dermatitis (9–11). These findings provide further clinical evidence for a role of exposure to inhalant allergens in the pathogenesis of atopic dermatitis. In the present study we chose the birch pollen antigens in addition to house dust mite for the patch tests. Unlike house dust mite, the birch

Table II. *Positive keratinocyte staining for HLA-DR and CD1 antigens in biopsies from positive epicutaneous test reactions to inhalant allergens*

Patient	Allergen	Time (h)	Antigen	
			HLA-DR	CD1
1	Birch pollen	72	+	+
	House dust mite	72	+	+
2	Birch pollen	72	–	+
	House dust mite	72	+	+
3	Birch pollen	48	+	+
4	Birch pollen	48	+	+
	Birch pollen	72	–	+
5	Birch pollen	72	+	+

pollen antigens provide the possibility to study patients in an allergen-free environment for most of the year. Our observation of a group of patients with birch pollen allergy of the immediate type who also show a seasonal exacerbation of the atopic dermatitis during the birch pollen season in spring, suggests that inhalant allergens may play a role in the pathogenesis of atopic dermatitis. The present study included four such patients, three of whom showed a positive delayed-type reaction to birch pollen.

The HLA-DR expression of keratinocytes is induced by gamma interferon present in the lymphocytes underlying the epidermis and possibly in the epidermis itself (12). Barker and co-workers have shown that allergic contact dermatitis is followed by HLA-DR expression of keratinocytes (13, 14). These authors did not find similar HLA-DR expression in the keratinocytes in natural lesions of atopic dermatitis. This was taken as evidence that atopic dermatitis differs from allergic contact dermatitis and hence is not a delayed type of hypersensitivity response. In contrast, we found HLA-DR positive keratinocytes in most of the positive patch test reactions. Recently similar results have been reported for natural lesions of atopic dermatitis (15). Keratinocytes displaying HLA-DR can, however, be seen in many other inflammatory conditions with underlying T cell infiltrates (16, 17). Similarly keratinocytes displaying CD1 have been observed in various dermatoses (18).

In conclusion, some patients with atopic dermatitis have delayed type hypersensitivity reactions to inhalant allergens and these reactions may be clinically relevant (3, 15). However, the precise mechanism of these reactions is still unclear.

ACKNOWLEDGEMENTS

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Intracutaneous Tests with Pityrosporon Extract in Atopic Dermatitis

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In patients with atopic dermatitis and in a control group prick and intracutaneous tests were performed with Pityrosporon extracts. Moreover, specific IgE against Pityrosporon was determined in these patients. Significant differences existed between the results obtained with these methods in both groups of patients. Therefore, both skin tests with Pityrosporon extract and IgE determinations may contribute to a diagnosis of AD; moreover, it is likely that Pityrosporon may be of significance in the pathogenesis of AD.

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Yeasts of the genus Pityrosporon (*Malassezia furfur*) are known to constitute a major proportion of the saprophytic microflora of human skin and are associated with a number of disease conditions including pityriasis versicolor, folliculitis, seborrhoeic dermatitis, dandruff, acne vulgaris and systemic infections of compromised patients. Clemmensen and Hjorth (1) and Waersted and Hjorth (2) found evidence by means of prick tests and by treatment with ketoconazole that *Pityrosporon orbiculare* is of importance in several cases of atopic dermatitis, especially those patients with head and neck dermatitis.

Because no other atopic allergen is known to us giving an immediate type reaction only or almost only in patients with atopic dermatitis (AD), we intended to do a similar investigation. This was extended, however, to intracutaneous tests—which we believe sometimes to be more reliable especially in AD patients because of difficulties in the evaluation of the skin tests—and to specific IgE determinations by means of RAST for the same reason.

MATERIALS AND METHODS

Patients and controls

109 patients were skin tested (prick and/or intracutaneous tests); of these patients, 57 had AD according to the criteria of Hanifin and Rajka (3), 52 patients served as controls. The group of controls consisted of: (*a*) 41 atopic patients with asthma and/or rhinitis; 25 of these had one or more positive skin tests to atopic allergens such as housedust. *Dermatophagoïdes pteronyssinus*, pollen or animal danders. (*b*) 7 non-atopic patients with allergic contact dermatitis. (*c*) 4 non-atopic patients with seborrhoeic dermatitis or pityriasis versicolor. Specific and total IgE were determined in 34 patients with AD (diagnosis made according to the criteria of Hanifin and Rajka (3)) and in 10 atopic controls suffering from asthma and/or rhinitis.

Skin tests

Intracutaneous tests were performed with 0.05 ml of two different extracts of *Pityrosporon orbiculare:* extract A prepared by ALK (Copenhagen, Denmark) and extract B prepared in our own laboratory. Prick tests were performed with extract A only. All tests were read after 20 min, 6 hours and 24 hours. Moreover, all patients were tested with control solution, histamin HCl 1:100000, house dust 0.5% (HAL), *Dermatophagoïdes pteronyssinus* 10 U/ml (HAL), grass pollen 100 U/ml (HAL), tree pollen 100 U/ml (HAL), cat- and dog dander 0.01% (HAL). The results of the skin tests were graded 1+ through 3+; a 1+ reaction a wheal of no more than 5 mm diameter with erythema, a 2+ reaction a wheal of 5–8 mm with erythema and a 3+ reaction a wheal of 8–12 mm with pseudopods and erythema.

Pityrosporon extracts

A) Extract prepared by ALK (Copenhagen, Denmark); this extract was used in a concentration of 5 mg/ml in the prick test and 0.05 mg/ml in the intracutaneous test. B) Extract prepared in our own laboratory; the inoculating pure organism *Malassezia furfur=Pityrosporon ovale* was obtained through the Centraal Bureau voor Schimmelcultures (Baarn) from Gist-Brocades Inc. (Delft, The Netherlands).

A liquid medium was prepared containing NeoPeptone (Difco) 10 g, extract of baker's yeast (10 g) and D-glucose (20 g) per 1000 ml of distilled water. The solution was heat-sterilized and subsequently 50 ml of sterile olive oil was dispersed into the medium. The organism was cultured in this broth for 3 days at 37°C. The growth was removed by filtration and the culture filtrate was cleared of residual oil by centrifugation and prolonged dialysis. The culture filtrate antigens were finally obtained by lyophilization. This extract was used in a concentration of 0.5 mg/ml in the intracutaneous test.

Specific IgE

For direct RAST-testing, the culture filtrate antigens as described above were coupled to cellulose paper discs with cyanogen bromide by a modification of the method of Ceska, Eriksson and Varga (4). The RAST was further carried out with radioactive anti IgE, purchased from Pharmacia AB (Uppsala, Sweden). The results were expressed in percent

Table I. *Intracutaneous tests with Pityrosporon extract read after 20 min*

	Total no. of patients		No. of positive reactions	
Atopic dermatitis	40		35 (87.5%)	
Controls: rhinitis and asthma (positive reactions to inhalants)	25	41	5 (20%)	5 (12%)
Controls: rhinitis and asthma (negative reactions to inhalants)	16		0 (0%)	
Total	81		40 (49%)	

binding of added radioactivity to the allergen-coated discs. The maximum binding in this system was 45%.

Statistical calculations

For statistical analysis, all significance tests are based on a normal distribution (5).

RESULTS AND DISCUSSION

Skin test results in the patients with atopic diseases are presented in the Tables I and II. From these tables it is apparent that there is a statistically significant difference between the reactions after 20 min in patients suffering from AD and in patients with other atopic diseases ($p < 0.001$). In our group of 40 patients with atopic dermatitis tested intracutaneously, four patients reacted neither to Pityrosporon, nor to any inhalant allergens. If we exclude these four patients, as many as 34 of the remaining group of 36 patients (94%) reacted positively to the intracutaneous test

Table II. *Prick tests with Pityrosporon extract read after 20 min*

	Total no. of patients		No. of positive reactions	
Atopic dermatitis	44		26 (59%)	
Controls: rhinitis and asthma (positive reactions to inhalants)	25	34	0 (0%)	0 (0%)
Controls: rhinitis and asthma (negative reactions to inhalants)	9		0 (0%)	
Total	78		26 (33%)	

Table III. *IgE to pityrosporon*

	No. of patients	Elevated Pityrosporon IgE i.e. ≥ 2% binding
Atopic dermatitis	34	22 (65%)
Controls: rhinitis and asthma (positive reactions to inhalants)	10	0 (0%)

with Pityrosporon. No reactions were observed after 6 and 24 hours.

A group of 26 patients with AD was tested by both prick and intracutaneous tests with Pityrosporon extract A. As expected, the results showed that both methods in general produced qualitatively identical but quantitatively different results.

The results obtained with extracts A and B in the intracutaneous tests were almost always identical and have therefore not been presented separately.

Taking these findings together, one may conclude that with intracutaneous tests almost every patient with AD reacts to Pityrosporon, although some of the atopic controls also reacted. With prick tests all atopic controls are negative, but on the other hand a number of patients with AD are also negative.

Intracutaneous tests performed in 7 non-atopic patients with allergic contact dermatitis and in 4 non-atopic patients with seborrhoeic dermatitis or pityriasis versicolor produced negative results.

We could not find cross reactions in the skin test between Pityrosporon and possibly related allergens, for example *Candida albicans*.

In contrast with the findings of Waerstedt and Hjorth (2) we could not find any relation between the localization of the dermatitis and the result of the skin

Table IV. *Relation between total IgE and specific IgE to Pityrosporon in patients with atopic dermatitis*

Total IgE (kU/L)	Specific IgE (percentage of binding)			
	<2%	2–5%	5–10%	>10%
<1 000	7			
1 000–5 000	5	3	5	1
5 000–10 000		4	2	
>10 000		1	3	3
Total number of patients	12	8	10	4

test. In Table III the results are shown of the specific IgE determinations. As with the skin tests statistically significant difference ($p<0.001$) was observed between the AD patients and the atopic controls. In our patients with AD the highest values of specific IgE to Pityrosporon (up to 33% binding) were seen mostly in patients with high total IgE values. The relation between total and specific IgE is shown in Table IV.

High IgE values were generally seen in patients with generalized skin lesions. Again, there was no relation to skin lesions localized especially in the head-neck region, however. In our opinion all these data strongly point to a probable relationship between Pityrosporon and AD. To clarify further this point, more investigations are needed (e.g. isolation and enumeration of these yeasts from the skin in AD, patch-tests with Pityrosporon extract in patients with AD, specific therapy against Pityrosporon in these patients). Such investigations are now in progress in our Department.

Finally, skin tests with Pityrosporon extract and specific IgE determinations against this allergen may obviously contribute to the diagnosis of AD.

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Atopic Dermatits and House-dust Mites

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Atopic dermatitis (AD) is a disease, which may be caused by intrinsic factors including immunological- and pharmacological abnormalities and extrinsic factors such as food allergens and other common environmental allergens known as inhalant allergens and irritants.

Previous investigations have shown that AD patients have: 1) a high exposure to house dust mites; 2) that 75% of children with atopic diseases are sensitised to house-dust mites by the age of 10; that AD patients have a relative risk of 1.60 of positive skin tests to house-dust mites when born from May to November in Denmark; 4. patch testing with dust mite antigens for 48 h in superficially abraded skin sites may induce eczematous lesions in AD patients and the cumulative incidence rate has increased from 3% to 10% for the age interval 0–7 years for children born during the period 1960–1964 compared with 1970–1974.

Since the last investigation in 1985 concluded that new and presumable still unknown factors in the environment in our industrialized world must have affected the prevalence of the diseases, the purpose of the present study was to evaluate house-dust mites as possible etiologic agent in AD in a case-control study comparing the occurrence of house-dust mites in dwellings from patients and healthy controls

MATERIAL AND METHODS

Twenty-six AD patients without bronchial asthma were investigated. Eleven (5 men and 6 women aged 12 to 52 years (mean 23)) had mild AD and 15 (3 men and 12 women aged 16 to 38 (mean 27)) had moderate to severe AD according to the score system proposed by Rajka and Langeland.

A second group of 20 non-atopic patients with psoriasis were included as a group of patients with a high production of skin scales.

As a control group 41 healthy persons were investigated. These were randomly selected from the Municipal Register in Århus and were all living in the same geographic area as the AD patients.

In all the homes dust samples were collected during the period April to May 1987 by vacuum cleaning mattress surfaces and bedroom floor.

All AD patients were skin prick tested using 5 HEP (Histamine Equivalent Prick) extracts of Dermatophagoides pteronyssinus.

RESULTS

The median and inter quartile ranges (IQR) of the concentrations of house-dust mites in 0.1 g dust samples from the two locations in the three study groups are shown in Table I.

When moderate to severe AD patients were compared to the controls the exposure to house-dust mites in the mattresses corresponded to a relative risk of 4.6 and a clear dose-response relationship with an in-

Table I

	Atopic Dermatitis			
	Mild	Moderate	Psoriasis	Control
Mattress	1	85	19	8
IQR	(0–12)	(11–136)	(1–59)	(1–89)
P	NS	$p<0.01$	NS	
Bedroom floor	2	42	14	9
IQR	(0–18)	(11–116)	(0–152)	(1–115)
p	NS	NS	NS	
No	11	15	20	41

creasing relative risk with increasing exposure could be demonstrated.

The result of the prick test with house-dust mites showed 4 positive reactions among the patients with mild eczema and 8 positive in the other group.

CONCLUSIONS

There is a high concentration of house-dust mites in mattress dust in beds of atopic dermatitis patients with moderate to severe eczema.

The scale production did not seem to influence the concentration of house-dust mites in mattress dust.

The severity of eczema seems to depend on the concentration of mite antigen in the mattress dust.

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In Vitro Generation of IFN-gamma in Relationship to in vivo Concentration of IgE and IgG Subclasses and $Fc_\varepsilon R_L$/CD23 Positive Circulating Lymphocytes in Patients with Severe Atopic Dermatitis (AD)

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In fifteen patients with severe atopic dermatitis (AD) and ten healthy controls we investigated the in vitro generation of IFN-gamma and analysed the number of $Fc_\varepsilon R_L$/CD23 (low affinity Fc receptor for IgE) positive lymphocytes. We found a significantly impaired capacity to secrete IFN-gamma after PHA-stimulation compaired to controls in a significant proportion of patients. Serum IgG_4 levels in patients were higher compared to controls. A significant portion of lymphocytes bearing the low affinity Fc receptor for IgE (CD23) was observed with the moab Tü1 in patients. Lymphocytes from healthy donors were completely negative or $< 2\%$ positive for Tü1. Despite small numbers of patients a significant correlation was found between IFN-gamma generation in vitro and IgE serum concentration in patients, whereas the IFN-gamma generation and IgG_4 concentration were negatively correlated in the patient group. The number of $Fc_\varepsilon R_L$/CD23 positive lymphocytes in patients was positive correlated with the serum IgG_4 and IgE concentration and negative correlated with IFN-gamma generation in vitro. Our data suggest that a possible dysregulation of IFN-gamma, Interleukin 4 or other lymphokine production may be related to increased IgE and IgG_4 production.

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In contrast to normal controls peripheral blood mononuclear cells (PBMC) from patients with AD spontaneously synthesize detectable amounts of IgE in vitro. Furthermore abnormal suppressor-cell activity is well documented and IgE-specific helper factors released by T-cells from AD patients are involved in the pathogenesis of the disease (1). Recently, the low affinity Fc-receptor $Fc_\varepsilon R_l$) for IgE normally present only on a minor portion of monocytes, B cells, eosinophils and platelets has been identified with an increased number on circulating monocytes, T and B cells in AD. Defranc et al. (3) have shown that Il4 is able to induce FcR_L/CD23 on B-cells and Snapper (4) found that Il4 may enhance IgE and IgG_1 production of B-cells and may induce MHC class II expression on resting B-cells. IFN-gamma has been found to inhibit I14-mediated FcR_L/CD23 expression on B-cells and enhancement of IgE production. We have investigated the in vitro IFN-gamma production and analyzed the relationship to IgE and IgG_4 concentration in vivo and the number of FcR_L/CD23 positive circulating cells in patients with AD.

SUBJECTS

Fifteen patients with AD (median age 23 years, range 15–35 years) and ten healthy controls (median age 21 years, range 18–25 years) were investigated. The clinical degree of AD was evaluated by the criteria of Hanifin & Rajka (5).

METHODS

In vitro IFN-gamma production was induced by stimulating PBMC with phythaemagglutinin (PHA) at a dose of 1% stock-solution (Gibco, Karlsruhe, FRG). Cultures were performed in 24-well Costar culture plates (Tecnomara, Fernwald, FRG) at 1×10^6/ml for a period of 5 days at 37°C, 5% CO_2. The daily production of IFN-gamma was measured by removing the supernatants of activated PBMC. Supernatants were stored at −20°C until assayed for their content of IFN-gamma. Human IFN-gamma was assayed by an immunoradiometric assay from Celltech (Slough, UK) after 24 and 48 h stimulation of cells. Serum IgE concentrations were determined by a solid phase radioimmunoassay (Phadebas IgE Prist, Pharmacia, Freiburg, FRG) and IgG-subclasses were measured by enzyme linked immuno sorbent assay (ELISA) utilizing monoclonal subclass specific antibodies (Bio-Makor, Rehovot, Israel) as described by Jeffries et al. (6). PBMC labeled with moab (Tü1) were developed with a FITC-conjugated $F(ab)_2$ rabbit anti-mouse Ig (Dianova, Hamburg, FRG) as second antibody and analyzed on a FACS IV. Student's *t*-test was employed for statistical comparison and for correlation analysis the Spearman correlation coefficient was used.

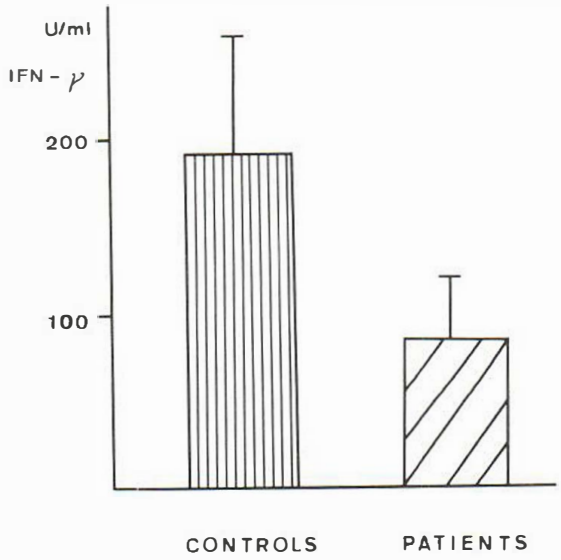

Fig. 1. IFN-gamma generation in vitro.

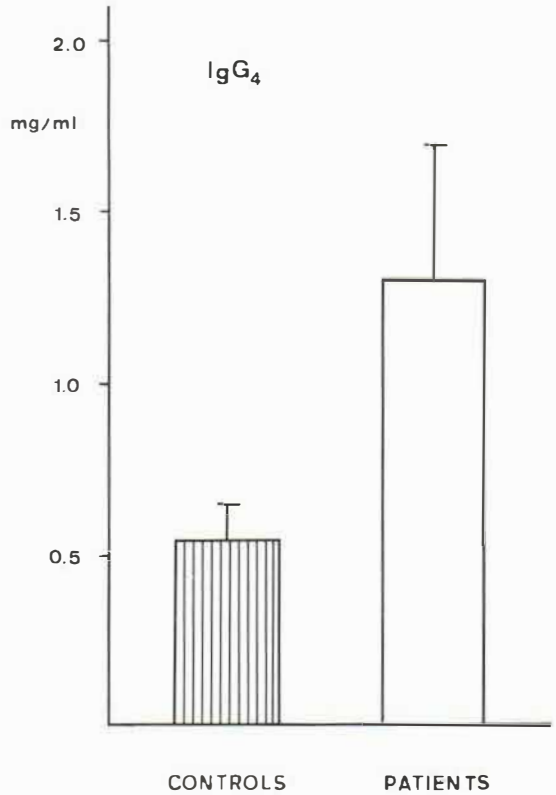

Fig. 2. IgG_4 serum concentration.

RESULTS

IFN-gamma generation by PBMC reached a maximum at 48 h after stimulation with PHA in AD and controls. IFN-gamma production in patients with AD (mean 90 ± 26 U/ml) was significantly lower ($p < 0.05$) than in controls (mean 193 ± 46 U/ml) (Fig. 1). The IFN-gamma levels in supernatants of unstimulated controls at 48 h were < 10 U/ml. The arithmetic mean of IgG subclass concentrations in controls did not differ significantly to WHO reference serum concentration (67/97). We found higher IgG_4 levels (mean 1.38 ± 0.40 mg/ml) in the patient group than in controls (0.59 ± 0.11), although this was not statistically significant due to low sample number ($n = 7$) (Fig. 2). Levels of IgG_1, IgG_2 and IgG_3 did not differ significantly from those of controls. In seven of eight patients a significant portion of Tü1-positive lymphocytes (range 2–10%) was observed, those of controls were completely negative or < 2%. Despite small numbers of patients a significant correlation was found between IFN-gamma generation in vitro and IgE serum concentration in vivo ($r = -0.66$, $p < 0.001$) (Fig. 3). The number of $Fc_\varepsilon R_L$/CD23 positive lymphocytes was positive correlated with serum IgG_4 concentration ($r = 0.97$, $p < 0.001$) (Fig. 4).

DISCUSSION

Our results demonstrate a highly significant relationship between in vitro and in vivo parameters in patients with AD. We found that PBMC of a significant proportion of patients have an impaired capacity to generate IFN-gamma after PHA-stimulation in vitro. IFN-gamma generation in vitro was significantly negative correlated with serum IgE concentrations in vivo. In agreement with other groups (6) we detected higher IgG_4 levels in sera of patients compared to controls.

Although the mechanism of IgG_4 elevation in AD is not clear, it has been suggested that it is raised due to prolonged exposure to an allergen which initiated an IgE response. Sherr et al. (1) have indicated a major role of helper factors released by activated T lymphocytes in the regulation of IgE secretion. Il 4 represents a T cell-derived lymphokine that enhances the secretion of IgG_1 and IgE and stimulates mast cell growth. Parkin et al. (8) suggested that the induction of $Fc_\varepsilon R_L$/CD23 on B cells seems to be specific for Il 4. Murine helper/inducer T cell clones are composed of at least two nonoverlapping subsets that can be distinguished on basis of their patterns of lymphokine secretion (9). The Th1 subset is able to produce Il2 and

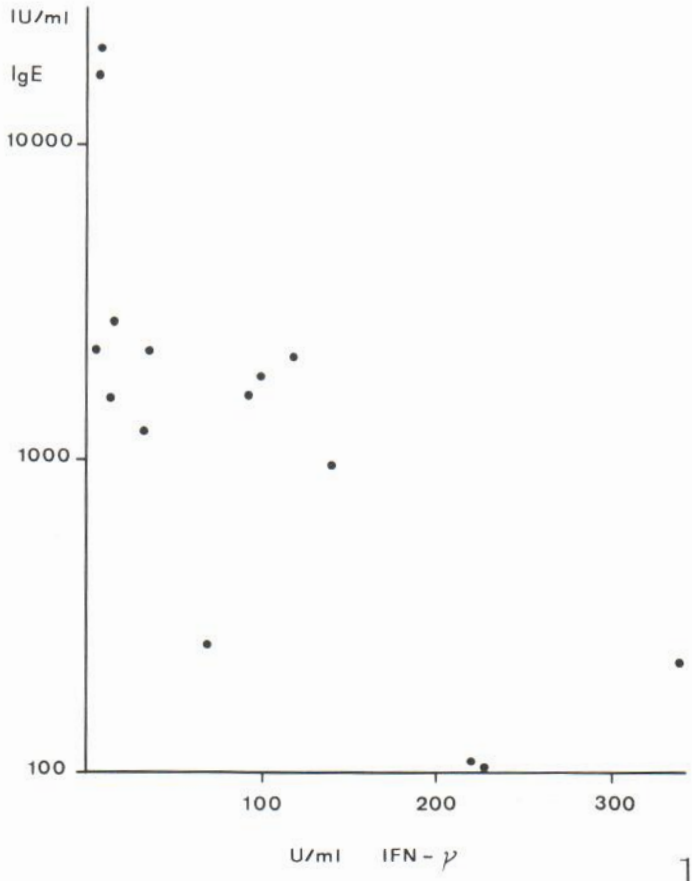

Fig. 3. Correlation between IFN-gamma generation in vitro and IgE serum concentration, $r = -0.66$; $p < 0.001$.

IFN-gamma in response to antigen receptor-mediated stimulation. The Th2 cell subset secretes Il 4 but not Il2. The T cell subset Th2 also secretes Il 5, which has been shown to have growth-promoting properties for eosinophils (10).

The selective activation of Th2 cells in vivo by certain allergic agents might be expected to increase $Fc_\varepsilon R_L$/CD23 expression, IgG_1 and IgE levels and the number of mast cells that bind IgE and mediate the allergic reaction. In addition Th2 cells may stimulate the growth of eosinophils via Il 5. Small quantities of IFN-gamma can totally inhibit the ability of Il 4 to stimulate B-cell growth, enhance IgG_1 and IgE production and the induction of $Fc_\varepsilon R_L$/CD23 on B cells (3). Our data suggest that the low IFN-gamma in vitro generation in a significant portion of AD patients may play a major role in the pathogenesis of increased IgE production and enhanced expression of $Fc_\varepsilon R_L$/CD23 lymphocytes. This is further confirmed by the observed correlation between IFN-gamma generation in vitro and serum IgE concentration in vivo.

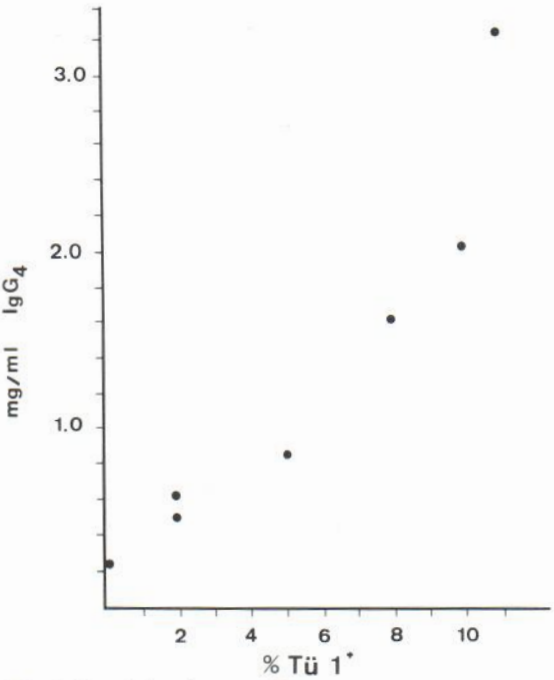

Fig. 4. Correlation between $Fc_\varepsilon R_L$/CD23 positive lymphocytes and IgG_4 serum concentration, $r = 0.97$; $p < 0.001$.

Studies of Strannegard (11) have indicated a defective capacity to generate interferons in response to viral antigens in AD. However, these and our results are in contrast to studies from Kapp et al. (12), who did not report any differences in IFN generation in AD patients in vitro. Interestingly, a portion of patients analyzed in their studies did not secret any or at least small levels of IFN-gamma in response to PHA which is actually in agreement with our present data. Furthermore in Kapp's study the IFN-gamma generation was measured using a bioassay while we used a highly sensitive IRMA-test specific for IFN-gamma in our experiments. Taken together the results of Kapp and our group suggest that defective IFN-gamma generation is not a primary defect in all AD patients, but is present in a subgroup of patients and seems to be an important factor in the pathogenesis of AD. Furthermore, the possible relation of in vitro parameters to the patients clinical status (all had severe AD) and course has to be elucidated in further studies.

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Atopic Dermatitis and the Indoor Climate

Preventive Measures Related to the Indoor Climate

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Atopic dermatitis (AD) is a disease, which may be caused by many factors. As a previous investigation concerning the increasing cumulative incidence rate of AD concluded that newer and presumably still relatively unheeded elements in the environmental must have affected the occurrence of the disease we tried to evaluate the atopic skin condition in a population of AD patient when moving to houses with the best indoor climate according to WHO i.e. a high air exchange rate, low humidity, and a subsequently lower allergen burden.

MATERIALS AND METHODS

Houses

In 1984 111 houses were built in Skejby, Jutland, Denmark. The houses were so-called "mini-risk" houses as the construction and choice of building materials were in accordance with present knowledge concerning best indoor environment. The buildings were equipped with a mechanical ventilation system which reduced the relative humidity by changing the indoor air once every hour. It removed the air from lavatories, kitchens and the sculleries, and supplied fresh prewarmed air to the living- and bedrooms.

Patients

Two groups of patients with various degrees of AD according to Hanifin, were selected from the out-patient clinic. One group composed of 9 AD patients (7 females, 2 males) were prepared to move house in October 1984. Their mean age was 23 years (range 3–47).

The other group consisted to the remainig AD patients who were not able to move to the "mini-risk" houses. Among these we selected the control group which was composed of 10 patients with AD (6 females, 4 males), mean age 24 years (range 3–43) matched according to age and degree of eczema. These control patients accepted not to move during the observation period (1984–1986) and they were instructed to keep the same furnishings during the investigation period. None of the patients had pets.

In general no changes in occupation or occupational environment took place within the observation period.

Evaluation

Both groups of patients were clinically evaluated in April 1984, 1985 and 1986 at the Department of Dermatology. At the evaluation in April 85 and 86 the mean time after changing residences was 4.2 and 15.8 months, respectively.

At the annual clinical evaluations the patients were checked weekly four times by the same investigator, who recorded localization, degree (mild, moderate, severe) and the extent of eczema. Erythema, vesicles, itching and lichenification were registered on a visual analog scale (VAS) ranging from no symptoms (0 mm) to severe symptoms (100 mm).

For one month each year, all patients made a daily overall VAS registration of their general subjective condition.

Indoor climate investigation

One day in April 1984, 1985 and 1986 we measured the concentration of particles (suspended and respirable), the air exchange rate, the relative indoor humidity, the temperature and the concentration of house dust mites (Dermatophagoides pteronyssinus) at the mattresses and bedroom floor.

For the "movers" the first indoor climate measurements were performed in the old houses in 1984 and thereafter in the "mini-risk" houses in 1985 and 1986.

RESULTS

Indoor climate investigation

All parameters measured in the dwellings ("movers" and controls) were within the limits recommended by WHO.

The indoor climate compared to the previous dwellings especially improved for air exchange rate and relative humidity (Table I).

The concentration of house dust mites were normal among the movers (median 2/0.1 g dust), but increased in the control group (median 21–25/0.1 g dust) (normal < 10/0.1 g dust). Temperature and concentration of dust (suspended, respirable) particles showed small variations.

PATIENTS

The clinical evaluation/subjective evaluation of the skin among the movers compared to the controls showed that the skin condition improved significant in the last part of the 16 months observation period after leaving the old dwellings. Itching of the dry atopic skin was expected to increase in the "mini-risk" houses where the humidity was lower, but 70%

Table I

	Movers		Controls		
	1984	1986	1984	1986	
Median value					
Air exchange rate/h	0.11	1.21 ($p<0.0005$)	0.22	0.29 ($p<0.05$)	($p<0.02$)
Relative humidity (%)	44	38 ($p<0.01$)	43	48 NS	($p<0.05$)

of the "movers" reported that the itching even decreased. The consumption of emollients was unchanged in 1984–1985 and decreased in 1985–1986. This may be due to a better general condition of the skin due to removal of irritants and allergens in the indoor climate.

The present investigation showed improvement in subjective symptoms and clinical status of AD patients after moving to "mini-risk" houses with an increased air exchange rate and low indoor humidity. No single factor in the indoor environment could directly be related to changes in the skin symptoms. The observations support the opinion that AD may be a multifactorial disease and that the indoor climate may be one of many factors governing the skin condition.

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Evaluation of the Hydration and the Water-holding Capacity in Atopic Skin and So-called Dry Skin

PER THUNE

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The hydration, the hygroscopicity and the water-holding capacity as well as the rate of water loss were measured in 1) dry non-eczematous skin in 13 patients (mean age 32 years) with atophic dermatitis (AD), 2) dry, xerotic skin of old patients (mean age 75 years) and 3) 17 controls (mean age 36 years) with normal skin and no history of AD. Significantly higher water flux was found in patients with AD compared to the other groups while all the other functional parameters were much lower. The obtained data suggest different functional disturbances in dry skin of patients with AD compared to old, dry (xerotic) skin. ***Key words: Hydration; Water loss; Atopic dermatitis; Old; Dry skin.***

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Skin dryness and itch are typical features of atopic dermatitis (AD) and it seems logical to relate both symptoms to abnormalities of the horny layer. While a low itch-threshold and prolonged itching could be objectively demonstrated several years ago (1), a low hydration of the stratum corneum combined with a high transepidermal water loss (TEWL) were only observed recently with newly developed technical devices (2). An increased rate of waterflux is characteristic of a defect barrier function and is typically recorded in AD and various scaly dermatoses (2, 3, 4).

The water-sorption–desorption test as developed by Tagami et al. (5) gives information about the hygroscopicity, i.e. the ability to take up water, and the water-holding capacity of the stratum corneum. While it was at first surmised that the former to a large extent was dependent on soluble, hygroscopic substances or the so-called "natural moisturizing factor" (NMF) components, which can be leached out of the epidermis by washing or treatment with aceton/-ether application of the skin, this theory has now been revised. It is believed that the intercellular lipids are of great importance for the water-holding state of the stratum corneum (6, 7). Besides this they have an important role in the cohesion of the corneocytes, in the barrier function, in protecting water soluble substances and they also contribute to a large extent to the plasticising properties of the stratum corneum (8).

Some studies have indicated that the hygroscopic properties are sooner restored than the water-holding capacity and the TEWL (5). These findings indicate that a normalization of the water-holding capacity requires a complete restoration of the stratum corneum, whereas the NMF components are restored or normalized earlier.

Clinically dry skin is an expression which needs objective evaluation. It is typically observed in patients with atopic dermatitis and old non-atopic patients with xerosis. It is of interest to investigate if the functional parameters related to the barrier function and the hydration are different in these two conditions. If so, this might indicate that there are different underlying pathogenetic mechanisms.

MATERIALS AND METHODS

The water-sorption–desorption test as described by Tagami et al. (5) was used in order to evaluate the water content, the hygroscopicity, i.e. the ability to take up water, and the water-holding capacity. Measurements were performed with the Corneometer CM 420 (3) on the upper arm before the application of water for 30 sec, 10 sec after blotting and at intervals of 30 sec for 2 minutes. The TEWL was measured with the evaporimeter as described by Nilsson (9). All measurements were performed at room temperature 19°–20°C and no ointment was applied to the area of measurement before the study.

Thirteen patients (mean age 32 years) who suffered from dry skin and otherwise fulfilled the criteria of AD were included in the study. The same measurements were also performed in 10 elderly subjects (mean age 75 years) who suffered from dry skin, and in 17 controls with normal skin and no history of atopy (mean age 36 years).

RESULTS AND DISCUSSION

In the present study the hydration values, the hygroscopicity and the water-holding capacity were significantly lower in the AD group than in both the controls and in the patients with dry, old skin (Fig. 1), indicat-

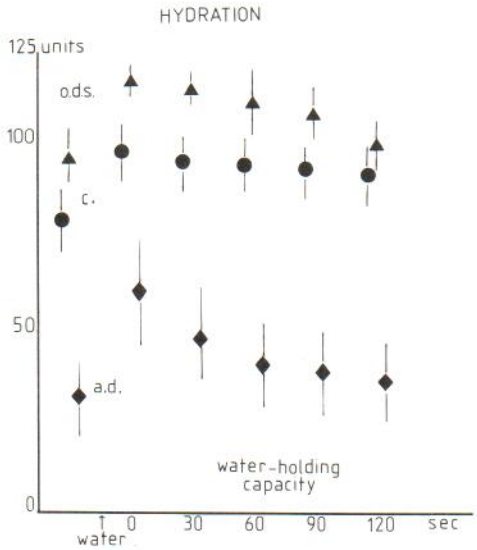

Fig. 1. Water sorption–desorption test showing the pre-hydration state, the hygroscopicity and the water-holding capacity in old, dry skin (o.d.s.), controls (c.) and atopic dermatitis (a.d.).

Table I. *Mean values of TEWL (g/m²/h) and hydration obtained in the present and a previous study*[a]

	TEWL	Hydration
Controls		
$n=23$*	8.3	70.5
Mean age 29 yrs	6.5–10.2	66.3–74.7
$n=15$*	4.6 ± 1.14	80.7 ± 2.7
Mean age 81 yrs		
$n=17$	4.8 ± 2.0	79.8 ± 16.9
Mean age 36 yrs		
Old, dry skin		
$n=40$*	6.9	71.8
Mean age 65 yrs	5.5–8.3	67.8–75.8
$n=10$	6.6 ± 4.4	86.5 ± 16.2
Mean age 75 yrs		
Atopic dermatitis		
$n=13$	20.0 ± 7.75	29.2 ± 21.3
Mean age 32 yrs		

[a] Data from REF (10).

ing a functional defect of the horny layer. An assumption which is supported by the observation that the TEWL values were clearly much higher in the AD group than in the two groups (Table I). For comparison are the values obtained in a previous study (10) included in the Table. One conclusion from the present study is that clinically dry-appearing skin in AD patients is really dry as measured with the Corneometer.

Previous reports on the water content of the stratum corneum in patients with AD and dry skin have shown conflicting data. This depends partly on the different methods applied, the different anatomical sites examined and probably also whether treated or non-treated skin was investigated. Two studies have indicated an increased water content of the uttermost portion of the stratum corneum in AD (11, 12). One was performed at the same anatomical site as the present one, but later these investigators (18) used the device of Tagami et al. (14) and came to the conclusion that there was no significant difference between the control subjects and patients with dermatitis in an inactive stage, including AD-patients. The present method measures mainly the deeper portions of the horny layer and the data are in accordance with those obtained by Werner (3) who used the same method. Also Tagami (5) came to the same conclusion regarding the water content, the hygroscopicity and the water-holding capacity in patients with various dermatoses including AD. It is apparent that the clinical state of the skin to be examined is of paramount importance since normal appearing skin in such patients show normal values. The high rate of water flux in AD-patients with dry skin is in concordance with the observations of others (2, 4). Interestingly, there are reports on the occurrence of abnormally small corneocytes and increased intracorneal cohesion in non-eczematous dry skin associated with AD (12, 13). All the findings are indicative of an abnormal barrier function.

The hydration data which were recorded in old, dry skin were higher but not significantly different from those observed in the control groups (Table I). This concerns also the hygroscopicity and the water-holding capacity (Fig. 1). Thus clinically dry appearing old skin is not always dry by measurement. On the other hand, by employing another in vivo technique which measures the propagation of shear waves through the skin, a lower moisture content in aged skin compared to young skin was suggested (15). Also in vitro measurements of the hygroscopicity and amount of bound water in samples of senile xerotic skin have indicated lower values than in normal horny layer (8).

The TEWL data from the patient groups with old, dry skin were quite similar. The same also holds true for the control groups with the exception of the youn-

ger ones which had a significantly higher rate of water flux. The reason for this discrepancy is not clear, but can probably be ascribed to the circumstances mentioned above concerning the hydration data in patients with AD.

Altogether, the present study indicates that the barrier function and the water-content in old, dry skin is not so much altered that it can be disclosed by the present techniques. The obtained data suggest different functional disturbances in old, dry skin compared to dry skin in patients with AD.

Since the skin is the primary site of involvement in AD it seems logical that more chemical and immunohistochemical research should be directed toward abnormalities in the epidermis, in particular the horny layer. Recent studies concerning intercellular lipid abnormalities combined with enzyme defect(s) (16) and the demonstration of Fc-R on Langerhans' cells from AD patients (17) are very promising in this respect.

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Randomised Double-blind Placebo-controlled Trial of Local Cyclosporin in Atopic Dermatitis

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The efficacy of local applications of 10% cyclosporin gel was studied in a randomised double-blind placebo-controlled trial in atopic dermatitis. Twenty atopic patients, presenting with stable symmetrical lesions, participated in the study. They applied cyclosporin gel to one side and placebo gel to the other side twice a day. The lesions were assessed on day 0 and day 14 according to lesional criteria scored from 0 to 3. The mean score for each criterion on day 14 was significantly lower on the sides treated with cyclosporin than on the sides treated with placebo. The total mean score on day 14 was 6.1 ± 3.9 on the sides treated with CSA and 9.6 ± 3.9 for placebo ($p < 0.005$). These findings suggest the efficacy of local CSA applications on lesions of atopic dermatitis.

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The therapeutic value of oral cyclosporin (CSA) in atopic dermatitis has already been reported (1). However, the risk of serious side effects associated with systemic administration and the return of active disease after stopping treatment limit the therapeutic possibilities of systemic administration.

Cyclosporin has a low degree of cutaneous penetration (2). However, the therapeutic action of local cyclosporin has been reported in experimental animals in a model of DNFP contact eczema and in man in the course of alopecia areata (3, 4, 5). In contrast, the local application of 2% cyclosporin does not appear to improve the lesions of psoriasis (6).

The aim of this study was to investigate the efficacy of 10% cyclosporin gel in patients with symmetrical lesions of atopic dermatitis by comparison of the effect of applications of CSA gel versus placebo gel applied to symmetrical lesions.

PATIENTS AND METHODS

Twenty patients, aged between two and twenty-nine years (mean: 11.6 ± 7.5 years), suffering from atopic dermatitis (diagnosis established on basis of the criteria defined by Hanifin & Rajka) and presenting with stable, symmetrical lesions (popliteal fossae, cubital fossae, extensor surface of the knees, wrists), participated in the study after giving their informed consent. The patients were supplied with tubes containing an alcoholic oily gel containing either 100 mg/g of cyclosporin or excipient alone.

The CSA gel and placebo gel were randomly allocated to the left and right sides. The patients applied the gel twice daily on the same zones for a period of 14 days and the lesions were assessed on day 0 and day 14 by the same observer for the following criteria: pruritus, erythema, vesicles and oozing, crusts, xerosis and lichenification. Each criterion was scored from 0 to 3.

Overall evaluation by the investigator on the 14th day was also scored according to 5 grades: complete cure, very good improvement, moderate improvement, no change, deterioration. The quantity of product used was determined by weighing the tubes at the end of the treatment period. Blood pressure was recorded on day 0 and day 14. A radioimmunoassay of cyclosporin was performed on a sample of whole blood at the end of treatment; the data were analysed statistically by Wilcoxon's test.

RESULTS

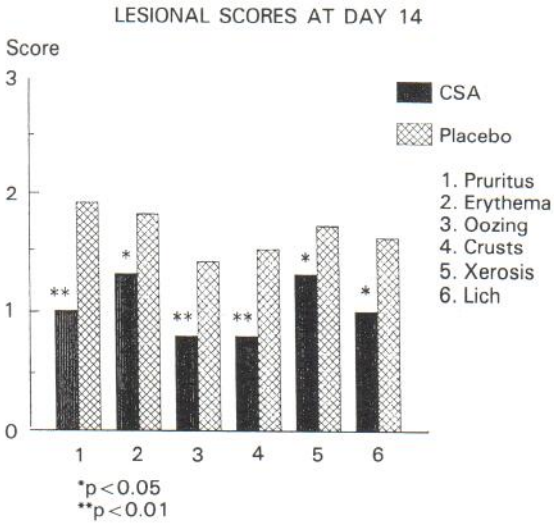
Eighteen patients completed the trial. One patient was lost to follow-up for intercurrent disease (frac-

Table I. *Baseline lesional scores*

	Right	Left
Pruritus	2.8 ± 0.5	2.8 ± 0.5
Erythema	2.5 ± 0.7	2.4 ± 0.8
Oozing	1.5 ± 1.0	1.5 ± 1.1
Crusts	2.1 ± 0.8	2 ± 0.9
Xerosis	2.9 ± 0.3	2.9 ± 0.3
Lichenification	2.2 ± 1.0	2.3 ± 1.0
Total score	13.9 ± 1.8	13.9 ± 1.9

Table II. *Results at day 14*

	CSA	Placebo	Comparison
Pruritus $n=18$	1.0 ± 0.7	1.9 ± 1.0	$p<0.005$
Erythema $n=18$	1.3 ± 0.9	1.8 ± 1.0	$p<0.05$
Oozing $n=16$	0.8 ± 0.9	1.4 ± 1.0	$p<0.01$
Crusts $n=17$	0.8 ± 1.1	1.5 ± 1.1	$p<0.01$
Xerosis $n=18$	1.3 ± 0.7	1.7 ± 0.6	$p<0.05$
Lichenification $n=18$	1.0 ± 0.9	1.6 ± 0.6	$p<0.05$
Total score $n=18$	6.1 ± 3.9	9.6 ± 3.9	$p<0.005$



tured leg) and one patient dropped out of the trial on the sixth day because of severe local irritation with burning sensations occurring on both sides after application.

The initial severity of the lesions was identical on the two sides for all of the criteria (Table I). The comparison of each criterion at the end of treatment revealed a statistically significantly greater improvement for all criteria in the CSA group compared with the placebo group (Tables II and III). The most marked difference concerned the criteria of pruritus, vesicles and crusts. The mean overall score on day 14 was 6.1 ± 3.9 in the CSA group versus 9.6 ± 3.9 in the placebo group ($p<0.005$). No cases of complete resolution of the lesions were observed in either group.

The overall evaluation by the investigator after treatment revealed 11 marked improvements in the CSA group compared with 2 in the placebo group (Table IV). No complete cures were observed.

Fifteen patients presented with minor side effects in the form of burning and prickling occurring several minutes after application of the product, on both sides in 12 cases and only on the side treated by placebo in 3 cases. These side effects were minor except in one patient in whom treatment had to be discontinued because of the severity of the irritation. Blood cyclosporin levels were always below the limit of detection (60 ng/ml). No variation in blood pressure was observed between the start and the end of treatment. The quantity of product used was 12.8 ± 7 g for cyclosporin and 13.5 ± 7 g for placebo.

DISCUSSION

The present investigation demonstrates the therapeutic efficacy of local application of 10% cyclosporin gel compared with placebo. The improvement in all of the criteria was significantly superior at the sites treated with cyclosporin in comparison to the sites treated with placebo.

The improvement of the site treated with placebo is a well-known phenomenon, probably related to the anti-inflammatory action of oily excipients. No cases of complete resolution were observed in either group. Before decoding the randomisation, we decided to define a group of responders when the difference between the side treated with placebo and the side treated with cyclosporin was equal to or greater than 3. According to these criteria, 10 patients out of 18 were considered to be responders (55%). Treatment was always well tolerated systemically. The local side effects, in the form of irritation and burning, were always transient, lasting several minutes after application of the product, except in one patient who had to

Table IV. *Global evaluation at day 14*

	CSA	Placebo
Good improvement	11	2
Moderate improvement	4	9
No change	3	6
Worsening	–	1

discontinue treatment. These effects occurred on both sides and were probably related to the alcoholic component of the excipient. The lymphocytic infiltrate present in the superficial dermis of the skin lesions of atopic dermatitis is essentially composed of helper T lymphocytes (7). The mechanism of action of cyclosporin in atopic dermatitis may involve inhibition of T cell proliferation and lymphokine secretion. These results encourage further therapeutic trials with better excipients ensuring better acceptability and better percutaneous penetration of cyclosporin.

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The Effect of Eicosapentaenoic Acid in the Treatment of Atopic Dermatitis. A Clinical Study

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There are two families of essential fatty acids, n-6 and n-3. Manipulation of dietary fatty acids in patients with atopic dermatitis (AD) have been restricted to the n-6 fatty acids. Leukotriene B4, a potent chemotactic agent derived from arachidonic acid (n-6), has been found increased in involved epidermis of AD (1).

Eicosapentaenoic acid (n-3) seems to substitute the arachidonic acid by competitive inhibition. We get leukotriene B5 which only possesses 3–12% of the biological activity of LTB4.

METHODS

31 patients with atopic dermatitis entered a 12-week double-blind block randomized trial. All patients were moderately or severely affected (2). Both the patients and the physician evaluated their symptoms on a scale from 0 to 10. Compliance was investigated by measuring the serum concentrations of phospholipids before and after the trial. The experimental group showed a significant elevation of n-3 fatty acids. The experimental group received 10 g of fish oil daily, of which about 1.8 g was eicosapentaenoic acid. The control group received 10 placebo (olive oil) capsules daily.

RESULTS

Patients' assessments showed that the fish oil (Max-Epa) was superior to placebo with regard to itch ($p<0.05$) and scaling ($p<0.05$). The total patients' symptom score showed greater improvement in the experimental groups as compared to the controls ($p<0.02$). The scores assessed by the physician showed no statistically significant difference between the groups. However, the total clinical scores evaluated by the physician was 30% higher in the experimental group than in the control group.

The amount of eicosapentaenoic acid consumed by the patients in the experimental group was not more than can be obtained from daily intake of fat fish. The beneficial effect of dietary n-3 fatty acids in the treatment of atopic dermatitis should be tested more extensively, using larger doses for a longer time.

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Recent investigations on the Relationship between Fungal Skin Diseases and Atopic Dermatitis

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Atopic dermatitis may be associated with chronic dermatophyte infections and Pityrosporum related disorders. Recent epidemiologic studies in school children and young recruits have confirmed that atopic individuals have an increased susceptibility to Trichophyton rubrum infections of the feet and an increased risk for persistant infections. In contrast, an investigation on skin reactivity in dermatophyte infected atopic patients indicated that a group of these patients is fully able to eliminate the fungi concomitant with the development of a delayed type skin reactivity. Facial erythema and scaling, often including neck and shoulders, is present in many young adults with atopic dermatitis. Preliminary data from a Danish-Swedish investigation have shown that atopic dermatitis patients with head-neck-shoulder dermatitis compared to a group without this disorder and normal individuals more often demonstrate positive prick test, RAST and specific histamin release using extract of Pityrosporum ovale. These findings indicate that the presence of Pityrosporum ovale in the skin may cause an allergic reaction leading to dermatitis.

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A multifactorial chain of different factors may cause exacerbations of atopic dermatitis (AD) including scratching, emotional stress, allergens, rapid temperature changes, exercise causing sweating, and microorganisms. Usually, the microorganisms related to are bacteria or virus, but recently it has been evident that also fungi i.e. Pityrosporum species may cause a deterioration of the disease (1). Lobitz et al. were the first to draw the attention to a correlation between AD and dermatophytosis (2, 3). Further studies showed that atopic patients were especially susceptible to chronic infections of the feet and hands caused by Trichophyton rubrum and at the first international symposium on AD in Oslo 1979 Jones suggested the term "the atopic-chronic dermatophytosis syndrome" (4). Thus, there may be two aspects of the relationship between fungi and atopic disease, an increased susceptibility to infection due to a compromized host defense and an exacerbation of the dermatitis caused by hypersensitization to the microorganisms.

ATOPIC DERMATITIS AND DERMATOPHYTOSIS

Previous epidemiologic studies showed that patients and families with chronic *T. rubrum* infections (CD) had a high incidence (40–70%) of atopic disease (5). A comprehensive study by Jones et al. showed that CD was three times more frequent in adults with AD than in non-atopic individuals (6). A recent study of tinea pedis in 15-year-old Danish school children showed a relative risk of 3 to contract tinea pedis in children with an atopic background (7). In a subsequent investigation of tinea pedis in Danish recruits examined before and after military service an increased susceptibility to tinea pedis in atopic individuals was not seen. However, an atopic predisposition was found in almost 50% of the soldiers with persistant infection at the final investigation (8).

Immunologic studies using intradermal application of trichophytin have previously shown that dermatophyte infected atopic patients react less often with a delayed type skin reaction than non-atopic patients. In a recent study on skin reactivity in atopic patients Kaaman (9) described two subgroups with different courses of dermatophyte infection. One group was characterized by the organisms *T. mentagrophytes, Epidermophyton floccosum* or *T. rubrum,* a short clinical course ending with cure and the presence of both delayed and immediate skin reactivity. Thirty percent of the patients in this group had atopic eczema without respiratory disease. The patients in the second group all had atopic respiratory disease, chronic *T. rubrum* infection and reacted with anergy or immediate type skin reactivity. The study indicates that AD patients infected with dermatophytes not necessarily develop CD, that this disease is related exclusively to *T. rubrum* and that atopic respiratory

Table I. *Frequency of positive prick test, RAST and Lucotest-HR using Pityrosporon ovale extract 5 mg/ml in 33 patients with atopic dermatitis complicated by erythematous scaling and itching dermatitis of head, neck and shoulders (HNS), 23 patients with atopic dermatitis without this disorder (AD), and 18 normal individuals without atopic dermatitis*

Group	No.	Calcofluor white* (% pos.)	Prick test** (% pos.)	Rast*** (% pos.)	Lucotest-HR**** (% pos.)
I HNS	33	77	79	24	70
II AD	23	18	44	4	48
III controls	18	0	6	6	0

HNS versus AD: $*p=0.006$; $**p=0.008$; $***p=0.05$; $****p=0.09$ not sign.

disease is a more important susceptibility factor than atopic eczema. The significance of the trichophytin reactivity in AD was studied by Rajka and Barlinn (10). The results suggested that immediate type reactions not necessarily mean sensitization to dermatophytes but may be a sign of cross reactivity to other moulds. Furthermore, that patients with CD without AD reacted even more frequently than the infected AD patients with an immediate type reaction indicating that other factors than AD play a role in the development of this type immune reaction in CD. Thus, recent studies have confirmed previous results concerning an increased susceptibility to persistent dermatophyte infection in atopic patients. However, it was also shown that the type of atopy i.e. respiratory plays a role and that a complete normal response to infection ending with cure may take place. Finally, it is noteworthy that CD in atopic patients usually is a restricted mild to moderate inflammatory condition indicating a relatively well functioning immune system compared to the widespread severe dermatophyte infections seen in the heavily immunocompromized patients with the acquired immune deficiency syndrome (AIDS).

ATOPIC DERMATITIS AND PITYROSPORUM SPECIES

Pityrosporum orbiculare/ovale (PO) are saprophytic lipophilic yeasts belonging to the normal microbial flora of the human skin. They mainly colonize the head, neck and upper part of the trunc. Various factors may cause the species to become pathogenic from simply the application of fatty lotions (11) to general immunosuppression during systemic corticosteroid treatment of AIDS. The disorders considered related to PO are Pityriasis versicolor, Pityrosporon folliculitis, confluent and reticular papillomatosis, seborrhoic dermatitis and psoriasis of the face and scalp (12).

In 1983, Clemmensen and Hjorth (1) reported on the benefit of ketoconazole in the treatment of atopic patients with a pronounced dermatitis of the head, neck and shoulders (HNS). They found many patients with AD to react positively to prick test with PO extract.

To further clarify the role of PO in AD and, in addition in seborrhoic dermatitis and psoriasis, an investigation including the history of the disease, a clinical description, identification of the fungus and immunologic studies was carried out as a co-operation between three dermatological departments. In this presentation in only the main preliminary results are given, as a more detailed report is under preparation (13). The first part of the study included group I, 33 AD patients with HNS, group II, 23 patients with AD without HNS, and group III, 18 control patients without atopy. In the clinical evaluation was used a simple score system, including grades of inflammation and area involved, location, diagrams and photo. Clinical involvement of head, neck and shoulders with scores higher than the remaining locations indicated HNS.

Identification of the fungus was done microscopically using calcofluor-white which makes the chitin-cellulose in the fungal membranes display an apple-green fluorescence in blue, ultraviolet or violet light. The material was skin scrapings taken by curette from the submandibular region.

The immunologic investigations included i) prick test with PO 5 mg/ml (ALK Laboratories, Denmark), ii) specific IgE antibodies against PO measured by RAST (ALK Laboratories, Denmark), iii) specific histamine release from basophilic leucocytes measured by Lucotest-HR (H. Lundbeck Diagnostics,

Denmark) (14), iv) total serum IgE (RIST), v) total leucocyte and different count, vi) T-lymphocyte ratio determination, vii) epicutaneous test with X-ray radiated PO and, viii) specific IgG antibodies against PO.

The most important preliminary data are given in the table. A positive microscopy means that a large amount of yeast spores were present per field of vision compared to normal skin, in which Pityrosporum is also present but in far less numbers. A more reliable quantitative method for the evaluation of the fungus is under investigation. Prick test and Lucotest-HR, an in vivo and an in vitro test for histamine liberation were both positive to a higher degree in the HNS group than in the pure AD group. The RAST test in which only allergy classes 3 and 4 were considered positive was excellent distinguishing the HNS from the pure AD group, but unfortunately gave many false negative results compared to the other methods.

Many of these patients were for a while treated with topical or systemic antimycotics with success, even relapses were observed after weeks to months. However, our investigations have shown that colonization of the HNS region in patients with AD may take place and cause a sensitization to the fungus leading to a flare of the eczema as an erythematosus scaling and itching dermatitis.

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Are Hyperlinear Palms and Dry Skin Signs of a Concomitant Autosomal Ichthyosis Vulgaris in Atopic Dermatitis?

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In 30% to 40% of cases atopic dermatitis (AD) is believed to be associated with autosomal dominant ichthyosis vulgaris (ADI). The diagnosis of ADI can be proved by the ultrastructural demonstration of a defective keratohyalin (KH) synthesis, resulting in minute granules of crumbly appearence in only one layer of granular cells. To investigate the suggested frequent association of ADI with AD, ultrastructural examination of dry skin of 49 AD patients was performed. Only in 2 patients abnormal KH was demonstrated by electron microscopy. 17 patients, including the 2 patients with abnormal KH, showed hyperlinear palms. The present study shows that hyperlinear palms and dry skin are in most cases a phenotypic marker of AD alone and not a sign of concomitant ADI. A histologically one-layered or absent stratum granulosum may occur in the dry skin of patients with only AD and does not indicate a manifestation of concomitant ADI in all cases. ***Key words: Ultrastructural analysis; Atopic dermatitis; Ichthyosis.***

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Atopic dermatitis (AD) and autosomal dominant ichthyosis (ADI) is believed to be frequently associated. The incidence of ichthyosis in atopic dermatitis ranges widely, from 2% to 40%, according to different studies (1–6). Some atopic features are seen in at least 50% of patients with ADI (7–9). Recent studies (2, 4–6) concluded that in 30% to 40% of the cases AD is associated with ADI. In these studies determination of the percentage of patients with both AD and ADI was based on clinical signs such as hyperlinear palms, keratosis pilaris and dry skin, as well as on the histological features of a reduced granular layer thickness (2, 4–6, 10). Depending on the time of year when the patients were examined, clinical features of ichthyosis vulgaris may be inapparent, but palmar hyperlinearity is usually a persistent finding. This feature has been described in one-third to one-half of patients with AD (10, 11).

Ultrastructural analyses of ADI performed by Anton-Lamprecht (12) demonstrated a severe disturbance of keratohyalin (KH) synthesis resulting in fewer and abnormal KH-granules. This abnormal KH is present in all ADI patients also in clinically unaffected skin (12–14). Thus, the defective KH of ADI can be used as a genetic marker to control the presence of the ADI gene (13).

In order to investigate the suggested frequent association ultrastructural analysis of AD patients was performed.

PATIENTS AND METHODS

Noneczematous but dry skin of 49 atopic patients (31 males, 18 females) aged 15–36 years was investigated using light and electron microscopy. The diagnosis of AD was established according to Haniffin and Rajkas (1). Further requirements for inclusion were a history of persistent dry skin and the absence of eczema on the lateral aspect of the buttock. All patients were examined for the presence of hyperliner palms (i.e., deep linear grooves crossing perpendicular to the thenar and/or hypothenar eminences (15)) and soles.

Punch biopsy specimens (4 mm) were taken from the skin of the upper outer quadrant of the buttock and fixed in 2.5% glutaraldehyde and postfixed with osmium tetraoxide. The samples were then dehydrated in a graded series of ethanol and then embedded in Epon 812. Semithin sections of 1–2 µm were cut and stained with 1% methylene blue and examined by light microscopy. Ultrathin sections were stained in uranyl acetate plus lead citrate prior to the examination in a JEOL 100 CX transmission electron microscope.

RESULTS

Light microscopic examination of semithin sections showed a normal thickness (16) of the granular layer

Table I. *Light microscopical examination*

Str. granulosum (layers)	Number of patients $n=49$
2–3	21
1–2	17
0–1	11

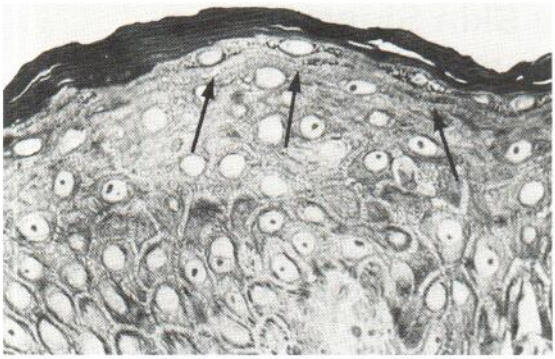

Fig. 1 a. Semithin section: two-layered str. granulosum (-->) in atopic dermatitis (×1 000).

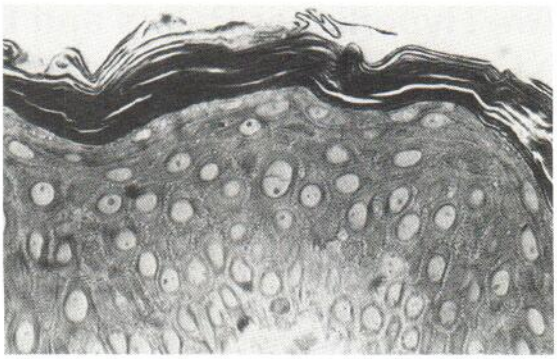

Fig. 1 b. Absence of str. granulosum in an atopic patient without ADI (×1 000).

(2–3 layers) in 21 patients and a thinned granular layer (1 or 1–2 layers) in 17 patients (Fig. 1 *a*), while 11 patients exhibited an absent or interrupted one-cell layer (Fig. 1 *b*, Table I). Electron microscopy demonstrated typical abnormal KH granules of crumbly appearence in only 2 patients (Fig. 2 *a*). The 9 remaining subjects with absent or interrupted stratum granulosum showed ultrastructurally normal KH (Fig. 2 *b*). Three patients had histological signs of eczematous inflammation with dermal infiltration of mononuclear cells. 17 patients (35%), including the patients with abnormal KH, showed hyperlinear palms.

DISCUSSION

The small percentage of patients with associated ADI (2 of the 49 AD patients (4%)) is in contrast with the findings of those investigators who did not perform ultrastructural analyses (4–7). Whether hyperlinear palms in a patient with AD is unique for AD or a manifestation of ichthyosis vulgaris has been unclear.

On the basis of ultrastructural investigations (3, 13) it seems most reasonable to assume that palmar hyperlinearity (ichthyotic hand) and dry skin (17) are in fact mainly traits of atopic dermatitis.

Though it has been widely believed that a one-layered or absent stratum granulosum indicates a concomitant manifestation of ADI, the present ultrastructural study clearly demonstrates that a one-layered or absent stratum granulosum in fact does not indicate a manifestation of concomitant ADI in all cases of AD. 9 subjects with a one-layered or absent stratum granulosum in light microscopy had ultrastructurally normal KH.

The reduction of KH may be due to the suppressive

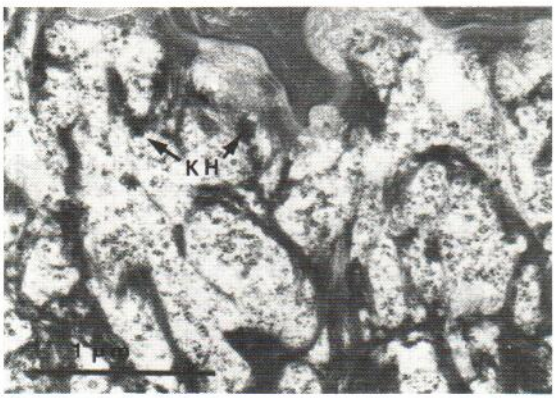

Fig. 2 a. Abnormal crumbly keratohyalin (KH) proving autosomal dominant ichthyosis vulgaris (ADI) in atopic dermatitis (AD) (×19 000).

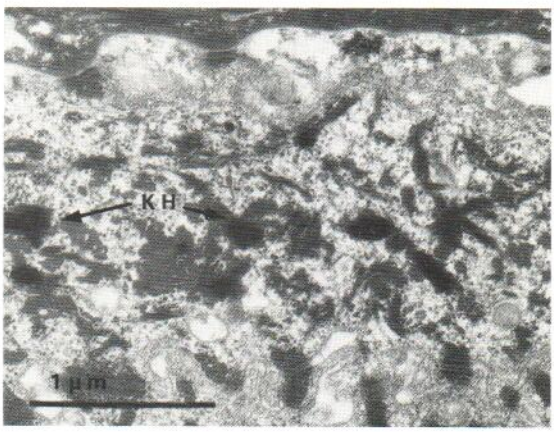

Fig. 2 b. Structurally normal but minute KH-granules, too small to be identified by light microscopy (×19 000).

influence of AD on keratinization and KH synthesis. This is supported also by the observation that bone marrow transplantation in children with Wiskott-Aldrich syndrome caused a simultaneous disappearance of AD and xeroxis associated with skin lesions (18). Thus, dry skin in AD appears, at least partly, to be related to the immune dysfunction and is not due to a primary defect of keratinization.

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Acta Derm Venereol (Stockh) 1989; Suppl 144: 146–148

Stuides on Dermographometry in Atopic Eczzema

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The usual method of investigating dermatographism (D), which is typically white in atopic eczema (AE), allows only a qualitative rating. To allow reproducible quantification of D we have developed an easily usable device termed Dermography. This can be fitted with one to three blunt tapered metal bars of different weight applying a constant stretching pressure over the whole skin area to be examined with different pressures at isolated lines. We used this device to study D in 27 patients with AE and in 20 healthy controls. Of the 27 patients, 21 had white D, 2 had red D, and 4 none at each pressure applied. In 18 of the 20 controls D was red. Both groups differed significantly with regard to the time until the onset of D and its duration, the former being prolonged and the latter shortened in patients with AE. Simultaneous and constant application of distinct grades of pressure for quantitative dermographometry is a method than can reliably be used for the study of inter- and intraindividual variations in vascular reactivity. ***Key words: Dermographism (white, red, indifferent); Latency time; Duration.***

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The usual method of examining dermographism (D), which is typically white in patients with atopic eczema (AE), allows only a qualitative rating (4, 5, 6, 7, 10, 11). To study this vascular phenomenon more closely it is necessary to use methods which can be standardized and used for quantitative measurements. Thirty years ago Reed et al. (8) constructed a wheelbarrow-like device loadable with sets of different weights, which generates a dermographic line when drawn over the skin. Although this tool allows the application of defined forces on the skin by the rotating wheel, it can not apply different weights simultaneously. Moreover, the device generates only pressure and not additional rubbing to the skin unlike the usual elicitation of D by stretching the skin with a spatula or a blunt pin head.

The imperfection of Reed's method motivated us to develop, together with the German firm Lasco Umformtechnik Inc. (Coburg), a device which allows a standardized and quantitative examination of D by simultaneous application of 1, 2 or 3 definite weights (9). The device is made of aluminium (own weight 440 g) and looks like a sledge with two parallel skids (distance 9.5 cm) for carrying up to three steel pounders with conical and blunt pointed ends (contact area 2.55 mm^2 each) (Fig. 1). When this tool called "Dermograph" is drawn over the skin of an individual with a speed of about 4 cm/sec (Fig. 2), the different pounders (weight 157 g, 285 g, 425 g) produce a stretching pressure of 62, 112, or 167 g/mm^2, respectively. The 25 mm distance between the stretching points can be extended to 50 mm by taking off the mid-pounder. The virtually frictionless mounting of the pounders on the sledgebow guarantees the simultaneous elicitation of D by quantitatively different forces thus enabling the exact evaluation of the dermographic skin reaction to defined stretching.

DERMOGRAPHOMETRIC STUDY

This report deals with the results of a preliminaty study using dermographometry in 27 inpatients suffering from severe atopic eczema (16 females, 11 males, age 17 to 47 years, mean age 21.6 years) and 20 non-atopic healthy control subjects (8 females, 12 males, age 22 to 42 years, mean age 25 years). The patients had not used corticoid treatment for at least 3 months prior to inclusion in the study. All examinations took place on the fourth or fifth day of the patients' hospitalization, between 1 p.m. and 3 p.m., at a constant room temperature of 22–23°C. The study was performed between January and May.

We performed the dermographometric procedure on the back of the laying subject, below the right scapula. The onset and ending of the dermographic reaction were time-recorded and the type of colour change (pink or red; no change, white) was evaluated during the whole period of examination.

There were two groups of patients, group A with lichenification of the tested area, and group B with only dry skin in the tested area. 15 patients belonged to group A, the remaining 12 to group B.

RESULTS

Table I demonstrates the colour type of D elicited in the different test groups. 21 out of the 27 patients showed white D, while none of the controls did. Red

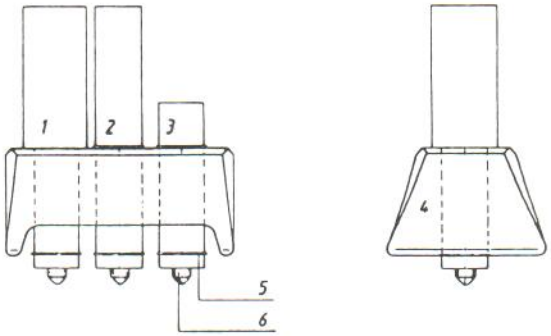

Fig. 1. Dermograph for manual use (schematic drawing). Sledge-shaped device carrying frictionlessly up to 3 pounders of different weight. Upper and lower holding rings prevent the pounders from slipping out the sledgebow. 1, 2, 3 = pounders; 4 = sledge; 5 = lower holding ring; 6 = blunt pencil-shaped end of pounder.

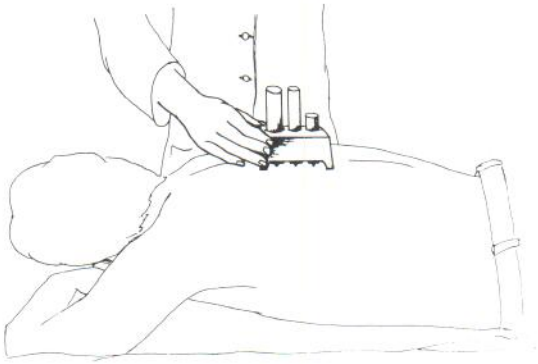

Fig. 2. Performance of dermographometry on the back of a laying subject.

D was observed in 2 patients of group B and in 18 of the 20 control subjects. Dermographic non-reactivity was found in two subjects of each group (group A, B and controls).

Table II shows the mean time values for the beginning and the disappearance of the dermographic reaction for the groups A, B, and the controls.

In comparison with the controls showing red D, the white D in the atopic subjects started later and faded away much earlier.

With increasing weight of the dermographic pounders both the latency time as well as the duration of white D was prolonged in the atopics. The red D in the control group lasted two to three times longer than the white D in the atopics. The white dermographic reaction of the atopics started earlier and lasted longer in lichenified skin than in the less severely affected dry skin. Two atopics in group B with short-lived red D demonstrated a very long latency time (60 or 105 sec, respectively) and a very short duration of D (only 3 min).

Table I. *Colour type of dermographism*

Dermographism[a]	Patients ($n=27$) Group A ($n=15$)	Group B ($n=12$)	Controls ($n=20$)
Pink or red	–	2	18
Unchanged	2	2	2
White	13	8	–

[a] Elicited with 3 different weights. Test area infrascapular, right side. Group A = lichenified skin, Group B = dry skin.

DISCUSSION

In the present study we compared the following items of dermographism (D) in atopic and non-atopic subjects:

1. Colour type of dermographic reactivity using simultaneous application of different dermographic weights. The results was that the colour type remained the same for the different weights simultaneously applied.
2. The comparison of the latency periods for normal and atopic dermographic reactivity demonstrated a remarkable starting delay of white as well as pink D in atopic subjects.
3. The comparison of the duration of dermographic reactivity in non-atopic and atopic subjects

Table II. *Dermographometry*

Onset (s) and Ending (m), mean values

		73 g/mm² O/E (s/m)	112 g/mm² O/E (s/m)	167 g/mm² O/E (s/m)
White	L ($n=13$)	15/15	22/16	38/19
	D ($n=8$)	35/16	50/16	50/19
Red	L ($n=0$)	–	–	–
	D ($n=2$)	60/3	105/3	105/3
Red	C ($n=18$)	6/29	7/47	7/60

L = lichenified skin; D = dry xerotic skin; C = controls; O = onset; E = ending; s = seconds, m = minutes.

showed a remarkable shortening of the dermographic time span in the atopics.

4. There was a positive correlation of latency time and duration of D to the stretching pressure applied in the atopics, but in the non-atopics only the duration of the dermographic reactivity was positively correlated to the strength of the stretching pressure. The latency time was shorter in lichenified skin than in non-lichenified skin of the atopics.
5. If the D was red, there was a longer latency and a much shorter total duration of the dermographic reactivity in the atopic subjects.

The explanation for the "paradoxical" white dermographism in patients with AE are a matter of controversy. Several authors regard the white type as the consequence of a superficial edema over dilated cutaneous vessels (2, 4, 10), whereas others assume an enhanced angiospastic reactivity of the cutaneous vessels (1, 3, 6, 7, 11). The results of current dermographiometric studies on another group of patients with AE are in favour of a moderate vasodilatation, since the infrared radiation emitted from the dermographed lines tends to increase slightly. However, additional microcirculatory measurements, such as cutaneous pO_2 pressure or microflowmetry in the dermographed area are required to clarify the overlapping conditions which are presumably operating in white dermographism.

By use of dermographometry we could demonstrate that the abnormal cutaneous reactivity in atopics tends to diminish parallell with the improvement of the eczematous skin condition. The quantification of D is a simple, reliable, easily repeatable and non-invasive method to study skin conditions in atopics. Moreover, the quantitative measurement of D provides a well reproducible method to examine the cutaneous microcirculatory reactivity in the course of many other inflammatory skin disorders.

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Responses of Skin Temperature to Different Thermic Stimuli in Atopic Eczema

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Patients suffering from atopic eczema (AE) often exhibit general disturbances of vasovegetative skin functions. Thus, in 21 patients with AE we studied the response of the skin of one forearm to standardized 15-min exposure of the other forearm to a moderate cold bath (17–18°C) and then, after a resting time, to a hot bath (40–41°C). The results were compared with those in 23 age- and sex-matched healthy controls under the same experimental conditions. In most patients, the unilateral skin exposure to warmth left the temperature of the contralateral forearm nearly unchanged or even slightly decreased, whereas the exposure to cold induced either a slight rise in contralateral skin temperature or only a minute decrease. In contrast to the normal consensual temperature reaction of the non-exposed forearm to warmth exposure of the contralateral arm in most controls, the results in atopic patients indicate a "rigid" or even inverse ("paradoxical") response to the thermic stimuli applied. This abnormal pattern of thermoregulation may reflect an intrinsic disturbance of the peripheral and hypothalamic autonomous neural system involved in the pathogenetic conditions of AE. *Key words: Thermoregulation; Abnormal reactivity, Hypothalamic dysfunction.*

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Many atopics are known for an abnormal cutaneous vasoactivity to temperature with vasoconstriction on warming and vasodilatation on cooling (2). Korting (5) noticed in the 1960s some signs of a disturbed cutaneous thermoregulation, and later Abrams and Farber (1) demonstrated, by means of plethysmographic investigations of the finger microcirculation, a weak vasomotoric response in the skin of atopic patients to variations in the room temperature. Kocsard et al. (4) studied the thermoregulatory behaviour of atopic subjects by immersing one hand in hot water of 41°C for 10 min and measuring the skin temperature on the contralateral side simultaneously. They found during the heat exposure not the normal consensual rise of the temperature but an inverse reaction on the other arm.

This "paradoxical" thermoregulatory inversion seems to indicate not only an alteration of the peripheral microcirculation but also a reflectory imbalance on the level of hypothalamic vasomotoric centres where afferent neuronal impulses are transmitted into efferent ones leading to a rapid consensual thermoreaction of the corresponding body site.

In recent neuro-endocrinological studies we found signs of an altered rhythm of the nocturnal secretion of melatonin and cortisol in a number of patients with atopic eczema (3, 6). We therefore felt that a dysregulation of subcortical or hypothalamic, resp., centres of the brain might be involved in the pathogenesis of atopic eczema (AE).

PATIENTS AND METHODS

We studied the thermoreactive behaviour of 21 patients with AE (9 males, 12 females, range of age 16–47 years, mean 23 years) on the 5th or 6th day of their hospitalization and 23 healthy control subjects (15 males, 8 females, ranging in age from 18–52 years, mean 28 years).

Each subject was seated in a climatized room with constant room temperature at 22–23°C for half an hour. None of the individuals were under corticoid treatment during or 3 months prior to the study, and none was allowed to smoke before the examination. The time of examination was between 1.00 p.m. and 3.00 p.m. All the tests were performed between January and May.

First, the right forearm was submerged in a bucket filled with cold water, tp. 17–18°C, for 15 min, and at the same time the skin temperature in the left elbow flexure was measured (Fig. 1) using a fine-calibrated contact thermometer (7), which records caloric differences of less than 0.1°C. Then, after an ample resting period of 90 min, the procedure was repeated using hot water, tp. 40°C, for the right forearm and measuring the skin temperature in the left arm flexure simultaneously.

RESULTS

Cold water exposure. 11 out of the 22 patients showed a fall of their consensual temperature between 0.3 and 1.5°C. In 3

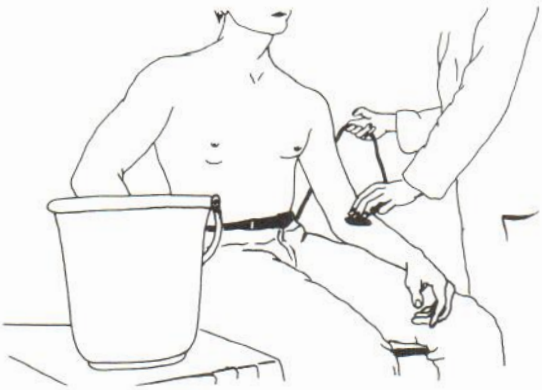

Fig. 1. Setting of consensual thermoregulatory experiments. Submersion of the right forearm in (cold or hot) water for 15 min, simultaneous contact thermometry at the opposite arm flexure.

patients the temperature increased, after an initial decrease, over that measured at the starting time (amplitudes from −1.4 to +0.9°C). In 5 patients after a slight initial rise, after 8 minutes a minor decrease of the temperature was recorded. All the changes in temperature, except in 2 patients, oscillated between −1°C and +1°C Fig. 2).

In contrast, 10 of the 23 control persons showed a more of less continuous rise of the temperature, and in the remaining ones a rather steady fall of the skin temperature was measured, in a few cases with a terminal reverse of the lowered values (Fig. 3).

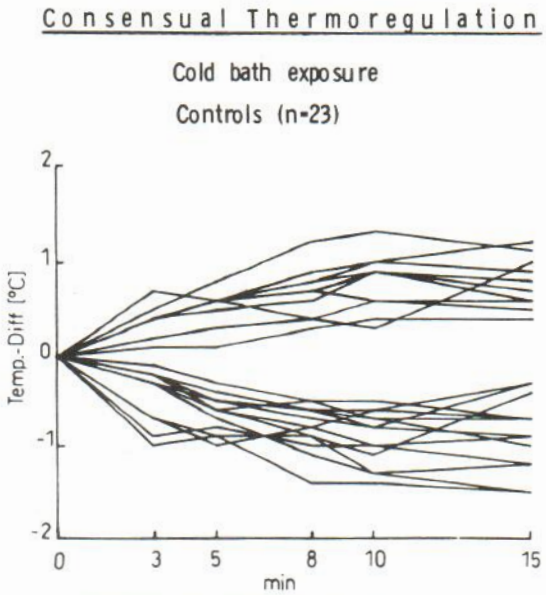

Fig. 3. Divergent skin temperature response to cold exposure in the control group.

Hence we found, in comparison to the controls, in the patient group the temperature profile to vary only slightly upon cold exposure, showing a "undecided" pattern which was apparent by the zigzag line of the course of the temperature curve.

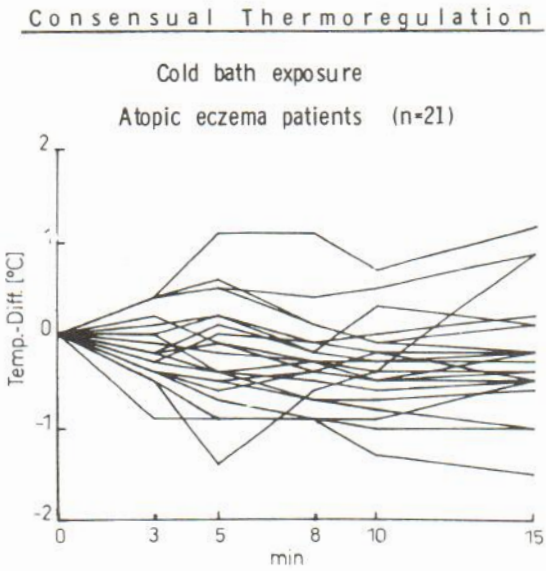

Fig. 2. Hyporeactive or inverse skin temperature response to cold exposure in the majority of AE patients. The ordinate indicates the initial values of all profiles as zero and the temperature differences related to this starting point.

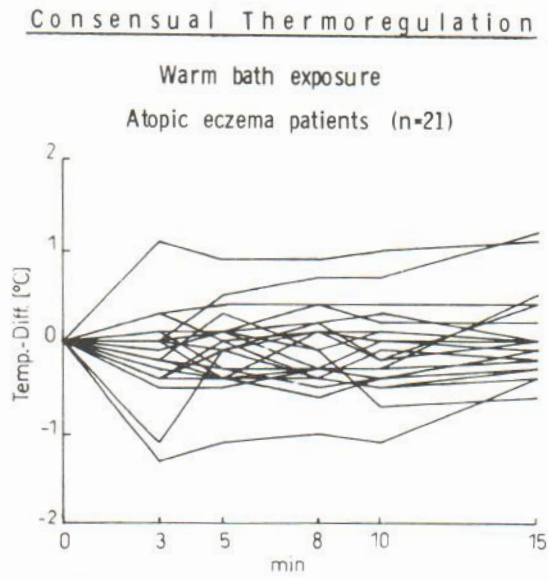

Fig. 4. Hyporeactive ("lazy") skin temperature response to heat exposure in most AE patients except a few showing either consensual or inverse temperature profiles.

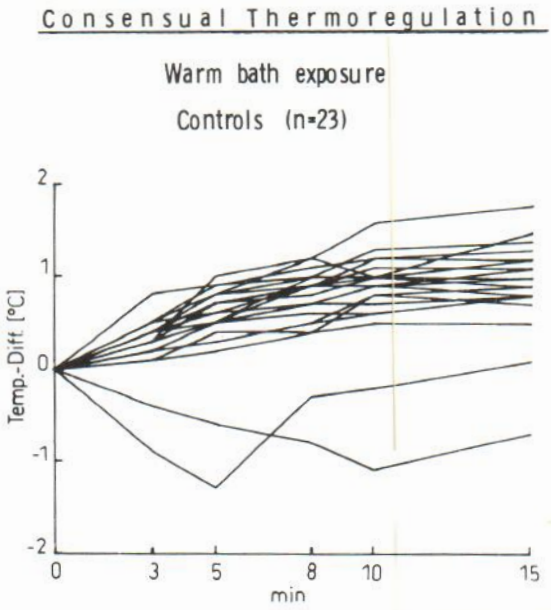

Fig. 5. Consensual skin temperature response to heat exposure in most controls except two subjects showing initial fall of temperature.

Hot water exposure. 4 out of the 22 patients exhibited an elevation of the temperature up to 1.2°C, yet in 8 the temperature fell till −1.3°C. Another 6 patients varied only slightly in their consensual skin temperature around the zero line (i.e., the continuation of the initial value). The remaining 3 patients showed more pronounced amplitudes of their temperatures, yet the values oscillated also in the vicinity of the zero line (Fig. 4).

Out of the 23 control subjects, 21 showed a continuous rise of their consensual temperature leading to values above 0.5°C after 10 min forearm exposure to hot water. In 2 subjects there was a remarkable fall of their temperature by −1.3°C after 5 min or −1.1°C after 10 min, resp., followed by a slight increase of the values (Fig. 5).

DISCUSSION

This study shows, as a common feature in the group of the patients, an altered regulation of the consensual skin temperature to cold and heat exposure. The thermographic pattern behaved inversely to cooling or heating of the contralateral forearm, or showed a rather "lazy" reaction to the thermic stimuli applied. Most patients reacted promptly to the initial caloric stimulus, but after 3–5 min exposure their skin temperature changed into a rather rigid or even "contrasensual" pattern.

How can these abnormal patterns of cutaneous thermoreactivity in patients with atopic eczema be explained?

The hypothalamic center for thermoregulation is maintaining the homeostatic "core" temperature of the body by inducing quick adaptations of the involved cutaneous mechanisms such as local blood flow, sweating, or muscle shivering, to exo- or endogenous caloric stimuli. Injuries to distinct nervous control centres, for example brain stem contusion, are known to alter also their thermoregulatory function. Minor deviations from the normal consensual response of the skin temperature to regional heat or cold exposure may also reflect an imbalance of the central thermoregulation, whatever the primary reason may be.

Thermoregulatory anomalies in patients with AE were first reported by Korting (5) and then by Kocsard et al. (4). In this study we have more extensely examined the feature by exposing the skin to different temperature stimuli. Interestingly, a few control subjects also showed an abnormal consensual response to temperature. They neither presented signs nor a history of atopy. An abnormal thermoreactivity is not specific for patients with AE, but it may indicate an intrinsic disturbance of both the peripheral and central autonomous nervous system involved in the pathogenetic conditions of AE.

In conclusion, in addition to local disturbances of the cutaneous microcirculation in patients with atopic eczema, there are also anomalies of the central thermoregulation in these patients. The abnormal thermoregulation to external cold and heat exposure is reflected by an either "lazy" or inverse ("paradoxical") consensual response to contralateral thermic stimuli. The anomalies of the thermoregulation in atopics with eczema may indicate a disturbance of the central vasomotoric control system possibly contributing to the pathogenetic conditions of atopic eczema.

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Juvenile Plantar Dermatosis (JPD)

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JPD is a frictional contact dermatitis of the forefoot to which atopics are prone but it also occurs in susceptible non-atopics. 189 affected individuals have been studied between 1973–1988.

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The term JPD (1) indicates a condition which usually begins over the plantar aspect of the big toes and then spreads to other toes and to the forefoot sole; toe spaces are unaffected but the heel may be involved. The condition has been described by others (2–7). Clinically there may be itching, red, dry, peeling, cracked, sore, painful, burning, bleeding, shiny skin and difficulty in walking.

MATERIAL AND METHODS

189 affected individuals (M = 108, F = 81 (M:F 1.3:1)) have been seen and studied personally.

FINDINGS

A study of 189 patients (1973–1988). M = 108, F = 81 (1.3:1). Age range of onset: Birth – 18 years (6 were < 1 year). Age range when seen: 15 months – 18 years 9 months. Mean age at onset: 6.8 yrs (M = 6.5/F = 7.2). Mean age when seen: 9.6 yrs (M = 9.2/F = 10.0).

Distribution apart from forefoot. 42/189 (22.2%) showed heel involvement.

Six pairs of siblings. This is a measure both of the high incidence of JPD plus what happens if you examine siblings! It neither indicates infectivity nor that JPD is a genodermatosis.

Month of referral.

Jan	Feb	Mar	Apr	May	June
14	19	12	20	15	15

July	Aug	Sept	Oct	Nov	Dec
17	24	10	12	16	15

Number of JPD patients seen each year (1977–88).

77	78	79	80	81	82	83	84	85	86	87	88
16	24	14	16	34	13	16	6	9	9	8	4 (up to 30 April)

Prognosis. A self-limiting condition, mean duration 7–8 years.

JPD and ATOPY.

Personal history of atopy	46 (24.3%)
Atopy in parent and/or sib	51 (27.0%)
Atopy in any relative	74 (39.2%)
Personal history and/or parent and/or sib	82 (43.4%)
Personal history and/or any relative	95 (50.3%)

Relationship to atopy. 95 (50.3%) of 189 patients seen since 1973 had a personal and/or family history of atopy. JPD may occur before, after, or at the same time as typical atopic dermatitis elsewhere. However, JPD behaves independently. Atopic dermatitis may also affect the foot of course, but can usually be distinguished from JPD as can allergic contact dermatitis.

DISCUSSION

It seems probable that the sequence of events in JPD is as illustrated below.

Juvenile plantar dermatosis

Occlusive footwear
↓
Oversweating
↓
Skin softening
↓
Pore blockage
↓
Anhidrosis
↓
Miliaria
↓
Frictional dermatitis

The basic requisite is occlusive footwear but it is unclear why the condition is self-limiting and why it predominates in juveniles. Leather shoes, open-toed shoes, cotton socks and emollients such as yellow soft paraffin and 10% urea in a cream are often helpful. Follow-up of patients has been described in many contributions (8–10).

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